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(54) **VARIANT THIOESTERASES AND METHODS OF USE**

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(56) **References Cited**

U.S. PATENT DOCUMENTS

4,049,724 A 9/1977 Sheng et al.
4,288,378 A 9/1981 Japikse et al.
4,335,156 A 6/1982 Kogan et al.
4,584,139 A 4/1986 Gray et al.
4,603,188 A 7/1986 Kusakawa et al.
4,683,202 A 7/1987 Mullis
4,798,793 A 1/1989 Eigtved
4,940,835 A 7/1990 Shah et al.
4,940,845 A 7/1990 Hirota et al.
4,945,050 A 7/1990 Sanford et al.
4,992,189 A 2/1991 Chen et al.
5,080,848 A 1/1992 Strauss et al.
5,091,116 A 2/1992 Krishnamurthy et al.
5,156,963 A 10/1992 Eigtved
5,233,099 A 8/1993 Tabata
5,233,100 A 8/1993 Tabata et al.
5,258,197 A 11/1993 Wheeler et al.

5,268,192 A 12/1993 Zook et al.
5,298,421 A 3/1994 Davies et al.
5,298,637 A 3/1994 Cooper
5,304,481 A 4/1994 Davies et al.
5,304,664 A 4/1994 Peppmoller et al.
5,342,768 A 8/1994 Pedersen et al.
5,344,771 A 9/1994 Davies et al.
5,346,724 A 9/1994 Ohgake et al.
5,380,894 A 1/1995 Burg et al.
5,391,383 A 2/1995 Sullivan et al.
5,427,704 A 6/1995 Lawate
5,434,278 A 7/1995 Pellosso et al.
5,451,332 A 9/1995 Lawate
5,455,167 A 10/1995 Voelker et al.
5,458,795 A 10/1995 Lawate
5,475,160 A 12/1995 Singleton et al.
5,506,201 A 4/1996 McDermott et al.
5,512,482 A 4/1996 Voelker et al.
5,567,359 A 10/1996 Cassidy et al.
5,576,027 A 11/1996 Friedman et al.
5,639,790 A 6/1997 Voelker et al.
5,654,495 A 8/1997 Voelker et al.
5,667,997 A 9/1997 Voelker et al.
5,674,385 A 10/1997 Ivaschenko et al.
5,686,131 A 11/1997 Sato et al.
5,693,507 A 12/1997 Daniell et al.
5,723,761 A 3/1998 Voelker et al.
5,776,741 A 7/1998 Pedersen et al.
5,807,893 A 9/1998 Voelker et al.
5,833,999 A 11/1998 Trinh et al.
5,850,022 A 12/1998 Dehesh et al.
5,885,440 A 3/1999 Hoehn et al.
5,888,947 A 3/1999 Lambert et al.
5,910,631 A 6/1999 Topfer et al.
5,928,696 A 7/1999 Best et al.
5,942,479 A 8/1999 Frankenbach et al.
5,945,585 A 8/1999 Hitz et al.
6,020,509 A 2/2000 Weerasooriya et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CN 102 066 569 A 5/2011
CN 102 300 996 A 12/2011

(Continued)

OTHER PUBLICATIONS

U.S. Office Action, dated Jul. 16, 2015, issued in U.S. Appl. No. 13/797,733.

(Continued)

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(57) **ABSTRACT**

The present invention relates to variant thioesterases and their use in plants, e.g., to increase enzymatic activity and to promote increased production of mid-chain length fatty acids (e.g., 8 to 14 carbons) and at desired ratios. Further disclosed herein are methods of manufacturing renewable chemicals through the manufacture of novel triglyceride oils followed by chemical modification of the oils. Oils containing fatty acid chain lengths of C8, C10, C12 or C14 are also disclosed and are useful as feedstocks in the methods described herein.

14 Claims, 3 Drawing Sheets

Specification includes a Sequence Listing.

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

WO WO 2016/014968 A1 1/2016
 WO WO 2016/044779 A2 3/2016

OTHER PUBLICATIONS

U.S. Final Office Action, dated Dec. 14, 2015, issued in U.S. Appl. No. 13/797,733.

U.S. Office Action, dated Jul. 26, 2016, issued in U.S. Appl. No. 13/797,733.

U.S. Notice of Allowance, dated Sep. 21, 2016, issued in U.S. Appl. No. 13/797,733.

U.S. Office Action (Requirement for Restriction/Election), dated Jul. 12, 2016, issued in U.S. Appl. No. 14/167,908.

U.S. Office Action, dated Apr. 3, 2017, issued in U.S. Appl. No. 14/167,908.

U.S. Office Action, dated Jul. 22, 2015, issued in U.S. Appl. No. 13/837,996.

U.S. Notice of Allowance, dated Nov. 17, 2015, issued in U.S. Appl. No. 13/837,996.

U.S. Office Action (Requirement for Restriction/Election), dated Jul. 12, 2016, issued in U.S. Appl. No. 14/209,931.

U.S. Office Action, dated Jan. 26, 2017, issued in U.S. Appl. No. 14/209,931.

PCT International Search Report and Written Opinion dated Jun. 24, 2014 issued in PCT/US2014/013676.

PCT International Preliminary Report on Patentability and Written Opinion dated Aug. 13, 2015 issued in PCT/US2014/013676.

Database Geneseq [Online] (Jun. 6, 2000) "Bay C18:1 preferring acyl-ACP thioesterase protein from clone 3A-17.", retrieved from EBI accession No. GSP:AAY80558 Database accession No. AAY80558; and Database Geneseq [Online] (Jun. 6, 2000) "Bay C18:1 preferring acyl-ACP thioesterase protein.", retrieved from EBI accession No. GSP:AAY80559 Database accession No. AAY80559.

Database Geneseq [Online] (Nov. 2, 1995) "Camphor thioesterase.", retrieved from EBI accession No. GSP:AAR74148 Database accession No. AAR74148.

Database Geneseq [Online] (Oct. 26, 1996) "Cuphea C14:0-ACP thioesterase.", retrieved from EBI accession No. GSP:AAW02081 Database accession No. AAW02081.

Database Geneseq [Online] (Aug. 5, 2010) "U. californica fatty acyl-ACP thioesterase protein (without PTS), SEQ:139.", retrieved from EBI accession No. GSP:AYC84249 Database accession No. AYC84249.

European Examination Report dated Oct. 25, 2016 issued in EP 14 706 996.7.

Mexican Office Action [no translation] dated Sep. 21, 2015 issued in MX/a/2015/009730.

PCT Invitation to Pay Additional Fees and, Where Applicable, Protest Fee dated Jun. 18, 2014 issued in PCT/US2014/026644.

PCT International Search Report and Written Opinion dated Aug. 29, 2014 issued in PCT/US2014/026644.

PCT International Preliminary Report on Patentability dated Sep. 24, 2015 issued in PCT/US2014/026644.

Genbank Accession No. U17097, *Umbellularia californica* UC FatB2 (FatB) mRNA, complete cds., Jun. 1, 1995, 2pp.

Genbank: Accession No. U39834.1, *Cuphea hookeriana* 8:0- and 10:0-ACP specific thioesterase (FatB2) mRNA, complete cds, May 21, 2014, 2pp.

Genbank Accession No. AAC49001, UC FatB2 (FatB) *Umbellularia californica*, May 30, 1995, 2pp.

European Partial Supplementary Search Report (Communication pursuant to Rule 164(1)EPC) dated Jul. 6, 2016 issued in EP 14 76 9502.7.

European Extended Search Report dated Oct. 13, 2016 issued in EP 14 76 9502.7.

PCT International Search Report and Written Opinion dated Dec. 22, 2015 issued in PCT/US2015/042044.

PCT International Preliminary Report on Patentability dated Feb. 2, 2017 issued in PCT/US2015/042044.

Database UniProt [Online] (Jul. 24, 2013) "SubName: Full =FatB type acyl-ACP thioesterase-3 {EC0:0000313:EMBL:AGG79285.1}," retrieved on Nov. 10, 2015 from EBI accession No. UNIPROT:R4J2L6, Database accession No. R4J2L6 sequence, 1 page.

Database UniProt [Online] (Jul. 9, 2014) "SubName: Full= Uncharacterized protein {EC0:0000313:EMBL:KCW58039.1}," retrieved on Nov. 16, 2015 from EBI accession No. UNIPROT:A0A059AWB4, Database accession No. A0A059AWB4 sequence, 1 page.

PCT Invitation to Pay Additional Fees and, Where Applicable, Protest Fee dated Jan. 13, 2016 issued in PCT/US2015/051042.

PCT International Search Report and Written Opinion dated Mar. 31, 2016 issued in PCT/US2015/051042.

Apt et al., (1996) "Stable nuclear transformation of the diatom *Phaeodactylum tricorutum*," *Molecular and General Genetics*, 252:572-579.

Barnes et al., (2005) "Contribution of 5'- and 3'-untranslated regions of plastid mRNAs to the expression of *Chlamydomonas reinhardtii* chloroplast genes," *Mol Gen Genomics* 274:625-636.

Blatti et al., (Sep. 2012) "Manipulating Fatty Acid Biosynthesis in Microalgae for Biofuel through Protein-Protein Interactions," *PLoS ONE* 7(9):e42949, 12pp.

Blowers et al., (Jan. 1989) "Studies on *Chlamydomonas Chloroplast* Transformation: Foreign DNA Can Be Stably Maintained in the Chromosome," *The Plant Cell*, 1:123-132.

Bonaventure et al., (Apr. 2003) "Disruption of the FATB Gene in *Arabidopsis* Demonstrates an Essential Role of Saturated Fatty Acids in Plant Growth," *The Plant Cell* 15:1020-1033.

Boynton et al., (1988) "Chloroplast Transformation in *Chlamydomonas* with High Velocity Microprojectiles," *Science*, 240(4858):1534-1538.

Chasan, (Mar. 1995) "Engineering Fatty Acids—The Long and Short of It," *Plant Cell*, 7:235-237.

Chen et al., (1988) "Recognition of prokaryotic transcription terminators by spinach chloroplast RNA polymerase," *Nucleic Acids Research*, 16(17):8411-8431.

Chen et al., (2001) "Highly efficient expression of rabbit neutrophil peptide-1 gene in *Chlorella ellipsoidea* cells," *Current Genetics*, 39(5):365-370.

Chow et al., (1999) "Electrotransformation of *Chlorella vulgaris*," *Plant Cell Reports*, 18:778-780.

Cobley et al., (Sep. 1993) "Construction of Shuttle Plasmids Which Can Be Efficiently Mobilized from *Escherichia coli* into the Chromatically Adapting Cyanobacterium, *Fremyella diplosiphon*," *Plasmid*, 30(2):90-105.

Cobley et al., (2002) "CpeR is an activator required for expression of the phycoerythrin operon (cpeBA) in the cyanobacterium *Fremyella diplosiphon* and is encoded in the phycoerythrin linker-polypeptide operon (cpeCDESTR)," *Molecular Microbiology*, 44(6):1517-1531.

Comai et al., (Oct. 15, 1988) "Chloroplast Transport of a Ribulose Bisphosphate Carboxylase Small Subunit-5-Enolpyruvyl 3-Phosphoshikimate Synthase Chimeric Protein Requires Part of the Mature Small Subunit in Addition to the Transit Peptide," *The Journal of Biological Chemistry*, 263(29):15104-15109.

Courchesne, Noémie Manuelle Dorval et al., (2009) "Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches," *Journal of Biotechnology*, 141(1):31-41.

Davies et al., (1992) "Expression of the arylsulfatase gene from the β_2 -tubulin promoter in *Chlamydomonas reinhardtii*," *Nucleic Acids Res.*, 20(12):2959-2965.

Dawson et al., (1997) "Stable Transformation of *Chlorella*: Rescue of Nitrate Reductase-Deficient Mutants with the Nitrate Reductase Gene," *Current Microbiol.*, 35(6):356-362.

Debuchy et al., (1989) "The argininosuccinate lyase gene of *Chlamydomonas reinhardtii*: an important tool for nuclear transformation and for correlating the genetic and molecular maps of the ARG7 locus," *EMBO Journal*, 8(10):2803-2809.

(56)

References Cited

OTHER PUBLICATIONS

- Dehesh et al. (1996) "Production of high levels of 8:0 and 10:0 fatty acids in transgenic canola by overexpression of Ch FatB2, a thioesterase cDNA from *Cuphea hookeriana*," *The Plant Journal*, 9(2):167-172.
- Dehesh et al., (1998) "KAS IV: a 3-ketoacyl-ACP synthase from *Cuphea* sp. is a medium chain specific condensing enzyme," *The Plant Journal*, 15:383-390.
- Deshnium et al., (1995) "Transformation of *Synechococcus* with a gene for choline oxidase enhances tolerance to salt stress," *Plant Mol. Biol.*, 29(5):897-907.
- Dörmann et al., (Jan. 1995) "Cloning and Expression in *Escherichia coli* of a Novel Thioesterase from *Arabidopsis thaliana* Specific for Long-Chain Acyl-Acyl Carrier Proteins," *Archives of Biochemistry and Biophysics*, 316(1):612-618.
- Dubois et al., (2007) "Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential," *Eur. J. Lipid Sci. Technol.*, 109:710-732.
- Eccleston et al., (1996) "Medium-chain fatty Acid biosynthesis and utilization in *Brassica napus* plants expressing lauroyl-acyl carrier protein thioesterase," *Planta*, 198:46-53.
- El-Sheekh et al., (1999) "Stable transformation of the intact cells of *Chlorella kessleri* with high velocity microprojectiles," *Biologia Plantarum*, 42(2):209-216.
- Facciotti et al., (1998) "Molecular dissection of the plant acyl-acyl carrier protein thioesterases," *Fett/Lipid*, 100(4-5, S.):167-172.
- Facciotti et al., (Jun. 1, 1999) "Improved stearate phenotype in transgenic canola expressing a modified acyl-acyl carrier protein thioesterase," *Nat Biotechnol.*, 17(6):593-597.
- Falciatore et al., (May 1999) "Transformation of Nonselectable Reporter Genes in Marine Diatoms," *Mar. Biotechnol.*, 1(3):239-251.
- Frenz et al., (1989) "Hydrocarbon recovery by extraction with a biocompatible solvent from free and immobilized cultures of *Botryococcus braunii*," *Enzyme Microb. Technol.*, 11:717-724.
- Fromm et al., (Sep. 1985) "Expression of genes transferred into monocot and dicot plant cells by electroporation," *Proc. Natl. Acad. Sci. USA*, 82:5824-5828.
- Ginalski et al., (2003) "Detection of reliable and unexpected protein fold predictions using 3D-Jury," *Nucleic Acids Research*, 31(13):3291-3292.
- Giuffrida et al., (2004) "Formation and Hydrolysis of Triacylglycerol and Sterol Epoxides: Role of Unsaturated Triacylglycerol Peroxyl Radicals," *Free Radical Biology and Medicine*, 37(1):104-114.
- Gruber et al., (1991) "*Escherichia coli*-*Anacystis nidulans* Plasmid Shuttle Vectors Containing the P_L Promoter from Bacteriophage Lambda," *Current Micro.* 22:15-19.
- Gruber et al., (1996) "Expression of the *Volvox* gene encoding nitrate reductase: Mutation-dependent activation of cryptic splice sites and intron-enhanced gene expression from a cDNA," *Plant Molecular Biology*, 31(1):1-12.
- Guo et al. (Jun. 22, 2004) "Protein tolerance to random amino acid change," *Proc. Natl. Acad. Sci. USA*, 101(25):9205-9210.
- Hall et al., (1993) "Expression of a foreign gene in *Chlamydomonas reinhardtii*," *Gene*, 124:75-81.
- Hallmann et al., (Nov. 1994) "Reporter genes and highly regulated promoters as tools for transformation experiments in *Volvox carteri*," *Proc. Natl. Acad. Sci. USA*, 91:11562-11566.
- Hanley-Bowdoin et al., (Feb. 1987) "Chloroplast promoters," *TIBS*, 12:67-70.
- Hawkins et al., (1999) "Expression of Human Growth Hormone by the Eukaryotic Alga, *Chlorella*," *Current Microbiology*, 38:335-341.
- Heise et al., (1994) "Factors Controlling Medium-Chain Fatty Acid Synthesis in Plastids From *Cuphea* Embryos," *Prog. Lipid Res.*, 33(1/2):87-95.
- Hejazi et al., (Apr. 2004) "Milking of microalgae," *TRENDS in Biotechnology*, 22(4):189-194.
- Hill et al., (1998) "Functional Analysis of Conserved Histidines in ADP-Glucose Pyrophosphorylase from *Escherichia coli*," *Biochem. Biophys. Res. Comm.*, 244(2):573-577.
- Hillen et al., (1982) "Hydrocracking of the Oils of *Botryococcus braunii* to Transport Fuels," *Biotechnology and Bioengineering*, XXIV:193-205.
- Hitz et al., (1994) "Cloning of a Higher-Plant Plastid ω-6 Fatty Acid Desaturase cDNA and Its Expression in a Cyanobacterium," *Plant Physiol.*, 105(2):635-641.
- Huang et al. (2006) "Expression of mercuric reductase from *Bacillus megaterium* MB1 in eukaryotic microalga *Chlorella* sp. DT: an approach for mercury phytoremediation," *Appl. Microbiol. Biotechnol.* 72:197-205.
- Inoue et al., (1994) "Analysis of Oil Derived From Liquefaction of *Botryococcus braunii*," *Biomass Bioenergy*, 6(4):269-274.
- Isbell et al., (Feb. 1994) "Acid-Catalyzed Condensation of Oleic Acid into Estolides and Polyestolides," *JAOCS*, 71(2):169-174.
- Jakobiak et al. (Dec. 2004) "The Bacterial Paromomycin Resistance Gene, aphH, as a Dominant Selectable Marker in *Volvox carteri*," *Protist*, 155(4):381-393.
- Jarvis et al. (1991) "Transient expression of firefly luciferase in protoplasts of the green alga *Chlorella ellipsoidea*," *Current Genetics*, 19:317-321.
- Jha et al., (2006) "Cloning and functional expression of an acyl-ACP thioesterase FatB type from *Diploknema (Madhuca) butyracea* seeds in *Escherichia coli*," *Plant Physiology and Biochemistry*, 44:645-655.
- Jiang et al., (Apr. 2005) "The Actin Gene Promoter-driven bar as a Dominant Selectable Marker for Nuclear Transformation of *Dunaliella salina*," *Acta Genetica Sinica*, 32(4):424-433.
- Jones et al., (Mar. 1995) "Palmitoyl-Acyl Carrier Protein (ACP) Thioesterase and the Evolutionary Origin of Plant Acyl-ACP Thioesterases," *The Plant Cell*, 7:359-371.
- Kalscheuer et al., (1999) "Establishment of a gene transfer system for *Rhodococcus opacus* PD630 based on electroporation and its application for recombinant biosynthesis of poly(3-hydroxyalkanoic acids)," *Applied and Environmental Microbiology*, 52:508-515.
- Kang et al., (Jul. 2000) "The Regulation Activity of *Chlorella* Virus Gene 5' Upstream Sequence in *Escherichia coli* and Eucaryotic Algae," [English Abstract] *Chinese Journal of Biotechnology*, 16(4):6 pages.
- Kang et al., (2004) "Genetic diversity in *Chlorella* viruses flanking kvv, a gene that encodes a potassium ion channel protein," *Virology*, 326(1):150-159.
- Kawasaki et al., (2004) "Immediate early genes expressed in chlorovirus infections," *Virology*, 318(1):214-223.
- Kim et al., (2002) Stable Integration and Functional Expression of Flounder Growth Hormone Gene in Transformed Microalga, *Chlorella ellipsoidea*, *Mar. Biotechnol.*, 4(1):63-73.
- Kindle, (Feb. 1990) "High-frequency nuclear transformation of *Chlamydomonas reinhardtii*," *Proc. Natl. Acad. Sci. USA*, 87(3):1228-1232.
- Klein et al., (1987) "High-velocity microprojectiles for delivering nucleic acids into living cells," *Nature London* 327(7):70-73.
- Knauf, (Feb. 1987) "The application of genetic engineering to oilseed crops," *TIBTECH*, 5:40-47.
- Knutzon et al., (Jul. 1999) "Lysophosphatidic Acid Acyltransferase from Coconut Endosperm Mediates the Insertion of Laurate at the sn-2 Position of Triacylglycerols in Lauric Rapeseed Oil and Can Increase Total Laurate Levels," *Plant Physiology*, 120:739-746.
- Kojima et al., (1999) "Growth and Hydrocarbon Production of Microalga *Botryococcus braunii* in Bubble Column Photobioreactors," *Journal of Bioscience and Bioengineering*, 87(6): 811-815.
- Koksharova et al., (Feb. 2002) "Genetic tools for cyanobacteria," *Appl Microbiol Biotechnol* 58(2):123-137.
- Krebbes et al., (1982) "The maize chloroplast genes for the β and ε subunits of the photosynthetic coupling factor CF₁ are fused," *Nucleic Acids Research*, 10(16):4985-5002.
- La Scala et al., (Jan. 2002) "The Effect of Fatty Acid Composition on the Acylation Kinetics of Epoxidized Triacylglycerols," *Journal of the American Oil Chemists' Society*, 79(1):59-63.

(56)

References Cited

OTHER PUBLICATIONS

- Lapidot et al., (May 2002) "Stable Chloroplast Transformation of the Unicellular Red Alga *Porphyridium* Species," *Plant Physiol.*, 129(1):7-12.
- Larson et al., (2002) "Acyl CoA profiles of transgenic plants that accumulate medium-chain fatty acids indicate inefficient storage lipid synthesis in developing oilseeds," *The Plant Journal*, 32(4):519-527.
- Lumbreras et al., (1998) "Efficient foreign gene expression in *Chlamydomonas reinhardtii* mediated by an endogenous intron," *Plant Journal*, 14(4):441-447.
- Manuell et al., (2007) "Robust expression of a bioactive mammalian protein in *Chlamydomonas* chloroplast," *Plant Biotechnol Journal*, 5:402-412.
- Mayer et al., (Feb. 4, 2005) "A Structural Model of the Plant Acyl-Acyl Carrier Protein Thioesterase FatB Comprises Two Helix/4-Stranded Sheet Domains, the N-terminal Domain Containing Residues That Affect Specificity and the C-terminal Domain Containing Catalytic Residues," *The Journal of Biological Chemistry*, 280(5):3621-3627.
- Mayer et al., (Jan. 3, 2007) "Identification of amino acid residues involved in substrate specificity of plant acyl-ACP thioesterases using a bioinformatics-guided approach," *BMC Plant Biology*, 7(1):1-11 pages.
- Mayfield et al., (Jan. 21, 2003) "Expression and assembly of a fully active antibody in algae," *Proc. Natl. Acad. Sci. USA*, 100(2):438-442.
- Mekhedov et al., (Feb. 2000) "Toward a Functional Catalog of the Plant Genome. A Survey of Genes for Lipid Biosynthesis," *Plant Physiology*, 122:389-401.
- Mendes et al. (2003) "Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae," *Inorganica Chimica Acta*, 356:328-334.
- Metzger et al., (Jun. 2003) "Lycopenoids I-L, Four New Tetraterpenoid Ethers from *Botryococcus braunii*," *J Nat. Prod.* 66(6):772-778.
- Metzger et al., (2005) "*Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids," *Appl Microbiol Biotechnol* 66:486-496.
- Miao et al., (2004) "High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*," *Journal of Biotechnology*, 110:85-93.
- Miao et al., (2006) "Biodiesel production from heterotrophic microalgal oil," *Biosource Technology*, 97:841-846.
- Minowa et al., (1995) "Oil production from algal cells of *Dunaliella tertiolecta* by direct thermochemical liquefaction," *Fuel*, 74(12):1735-1738.
- Mitra et al., (Oct. 14, 1994) "A *Chlorella* Virus Gene Promoter Functions As a Strong Promoter Both in Plants and Bacteria," *Biochemical Biophysical Research Communication*, 204(1):187-194.
- Mitra et al., (Oct. 1994) "The *Chlorella* virus adenine methyltransferase gene promoter is a strong promoter in plants," *Plant Mol. Biol.*, 26(1):85-93.
- Mittendorf et al., (1999) "Polyhydroxyalkanoate synthesis in transgenic plants as a new tool to study carbon flow through β -oxidation," *The Plant Journal*, 20(1):45-55.
- Moreno-Pérez et al., (2012) "Reduced expression of FatA thioesterases in *Arabidopsis* affects the oil content and fatty acid composition of the seeds," *Planta*, 235:629-639.
- Mullet et al., (1985) "Multiple transcripts for higher plant *rbcL* and *atpB* genes and localization of the transcription initiation site of the *rbcL* gene," *Plant Molecular Biology*, 4:39-54.
- Oda et al., (2000) "Degradation of Polylactide by Commercial Proteases," *Journal of Polymers and the Environment*, 8(1):29-32.
- Onai et al., (2004) "Natural transformation of the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1: a simple and efficient method for gene transfer," *Mol Genet Genomics*, 271(1):50-59.
- Park et al., (2005) "ICORBtion and Characterization of *Chlorella* Virus from Fresh Water in Korea and Application in *Chlorella* Transformation System," *The Plant Pathology Journal*, 21(1):13-20.
- Pröschold et al., (Aug. 2005) "Portrait of a species: *Chlamydomonas reinhardtii*," *Genetics*, 170:1601-1610.
- Radakovits et al., (Apr. 2010) "Genetic Engineering of Algae for Enhanced Biofuel Production," *Eukaryotic Cell*, 9(4):486-501.
- Rao et al., (2006) "Antioxidant Activity of *Botryococcus braunii* Extract Elucidated in Vitro Models," *J. Agric. Food Chem.*, 54(13):4593-4599.
- Rehm et al., (2001) "Heterologous expression of the acyl-acyl carrier protein thioesterase gene from the plant *Umbellularia californica* mediates polyhydroxyalkanoate biosynthesis in recombinant *Escherichia coli*," *Appl Microbiol Biotechnol*, 55:205-209.
- Rismani-Yazdi et al., (2011) "Transcriptome sequencing and annotation of the microalgae *Dunaliella tertiolecta*: Pathway description and gene discovery for production of next-generation biofuels," *BMC Genomics*, 12:148, 17 pages; doi:10.1186/1471-2164-12-148.
- Rosenberg, Julian N. et al., (2008) "A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution," *Current Opinion in Biotechnology*, 19(5):430-436.
- Salas et al., (Jul. 1, 2002) "Characterization of substrate specificity of plant FatA and FatB acyl-ACP thioesterases," *Archives of Biochemistry and Biophysics*, 403(1):25-34.
- Sanford, (Dec. 1988) "The biolistic process," *Trends in Biotech.* 6:299-302.
- Sawayama et al. (1999) Possibility of renewable energy production and CO₂ mitigation by thermochemical liquefaction of microalgae *Biomass and Bioenergy*, 17:33-39.
- Schreier et al., (1985) "The use of nuclear-encoded sequences to direct the light-regulated synthesis and transport of a foreign protein into plant chloroplasts," *EMBO J.* 4(1):25-32.
- Schultz et al., (Apr. 2005) "A common core of secondary structure of the internal transcribed spacer 2 (ITS2) throughout the Eukaryota," *RNA*, 11(4):361-364.
- Schütt et al., (1998) "The role of acyl carrier protein isoforms from *Cuphea lanceolata* seeds in the de-novo biosynthesis of medium-chain fatty acids," *Publication, Planta*, 205:263-268.
- Shao et al., (2002) "Cloning and expression of metallothionein mutant α -KKS- α in *Anabaena* sp. PCC 7120," *Marine Pollution Bulletin*, 45(1-12):163-167.
- Sheehan, John; Dunahay, Terri; Benemann, John; Roessler, Paul; (Jul. 1998) "A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae," Prepared for U.S. Department of Energy's Office of Fuels Development, Prepared by *National Renewable Energy Laboratory*, NREL/TP-580-24190, 328 pages.
- Stemmer et al., (1995) "Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides," *Gene*, 164(1):49-53.
- Tan et al., (Aug. 2005) "Establishment of a Micro-Particle Bombardment Transformation System for *Dunaliella salina*," *The Journal of Microbiology*, 43(4):361-365.
- Tang et al., (Aug. 1995) "Insertion Mutagenesis of *Chlamydomonas reinhardtii* by Electroporation and Heterologous DNA," *Biochemistry and Molecular Biology International*, 36(5):1025-1035.
- Tjellström et al., (Feb. 20, 2013) "Disruption of plastid acyl:acyl carrier protein synthetases increases medium chain fatty acid accumulation in seeds of transgenic *Arabidopsis*," *FEBS Letters*, 587(7):936-942.
- Tyystjärvi et al., (2005) "Mathematical modelling of the light response curve of photoinhibition of Photosystem II," *Photosynthesis Research*, 84(1-3):21-27.
- Vázquez-Bermúdez et al., (Jan. 2000) "Uptake of 2-Oxoglutarate in *Synechococcus* Strains Transformed with the *Escherichia coli* *kgtP* Gene," *Journal of Bacteriology*, 182(1):211-215.
- Vázquez-Bermúdez et al., (2003) "Carbon supply and 2-oxoglutarate effects on expression of nitrate reductase and nitrogen-regulated genes in *Synechococcus* sp. strain PCC 7942," *FEMS Microbiology Letters*, 221(2):155-159.

(56)

References Cited

OTHER PUBLICATIONS

- Voelker, (1996) "Plant Acyl-ACP Thioesterases: Chain-Length Determining Enzymes in Plant Fatty Acid Biosynthesis," *Genetic Engineering*, Edited by: Setlow JK. Plenum Pres, New York, 18:111-133.
- Voelker et al., (Dec. 1994) "Alteration of the Specificity and Regulation of Fatty Acid Synthesis of *Escherichia coli* by Expression of a Plant Medium Chain Acyl-Acyl Carrier Protein Thioesterase," *Journal of Bacteriology*, 176(23):7320-7327.
- Voelker et al., (1997) "Broad-Range and Binary-Range Acyl-Acyl-Carrier-Protein Thioesterases Suggest an Alternative Mechanism for Medium-Chain Production in Seeds," *Plant Physiol.*, 114:669-677.
- Voetz et al., (1994) "Three Different cDNAs Encoding Acyl Carrier Proteins from *Cuphea lanceolata*," *Plant Physiol.*, 106:785-786.
- Walker et al., (2005) "Characterisation of the *Dunaliella tertiolecta* RbcS genes and their promoter activity in *Chlamydomonas reinhardtii*," *Plant Cell Rep.* 23(10-11):727-735.
- Westphal et al., (Mar. 27, 2001) "Vipp1 deletion mutant of *Synechocystis*: A connection between bacterial phage shock and thylakoid biogenesis?" *Proc. Natl. Acad. Sci. USA*, 98(7):4243-4248.
- Wiberg et al., (2000) "The distribution of caprylate, caprate and laurate in lipids from developing and mature seeds of transgenic *Brassica napus* L.," *Planta*, 212:33-40.
- Wirth et al., (1989) "Transformation of various species of gram-negative bacteria belonging to 11 different genera by electroporation," *Mol Gen Genet.* 216(1):175-177.
- Wolk et al., (Mar. 1984) "Construction of shuttle vectors capable of conjugative transfer from *Escherichia coli* to nitrogen-fixing filamentous cyanobacteria," *Proc. Natl. Acad. Sci. USA*, 81(5):1561-1565.
- Wong et al., (1992) "*Arabidopsis thaliana* small subunit leader and transit peptide enhance the expression of *Bacillus thuringiensis* proteins in transgenic plants," *Plant Molecular Biology*, 20:81-93.
- Wu et al., (2001) "Identification of *Chlorella* spp. iCORBtes using ribosomal DNA sequences," *Bot. Bull. Acad. Sin.*42:115-121.
- Yu et al., (2011) "Modifications of the metabolic pathways of lipid and triacylglycerol production in microalgae," *Microbial Cell Factories*, 10:91 [Retrieved from the Internet Jul. 24, 2012: <URL:http://www.microbialcellfactories.com/content/10/1/91>], 11 pages.
- Yuan et al., (Nov. 1995) "Modification of the substrate specificity of an acyl-acyl carrier protein thioesterase by protein engineering," *Proc. Natl. Acad. Sci. USA*, 92:10639-10643.
- Yuan et al., (Feb. 16, 1996) "The Catalytic Cysteine and Histidine in the Plant Acyl-Acyl Carrier Protein Thioesterases," *The Journal of Biological Chemistry*, 271(7):3417-3419.
- Zurawski et al., (1981) "The structure of the gene for the large subunit of ribulose 1,5-bisphosphate carboxylase from spinach chloroplast DNA," *Nucleic Acids Res.* 9(14):3251-3270.
- Zurawski et al., (Dec. 1982) "Nucleotide sequence of the gene for the M_{32,000} thylakoid membrane protein from *Spinacia oleracea* and *Nicotiana debneyi* predicts a totally conserved primary translation product of M_{38,950}," *Proc. Natl. Acad. Sci. USA*, 79:7699-7703.
- U.S. Notice of Allowance, dated Jul. 10, 2017, issued in U.S. Appl. No. 14/167,908.
- U.S. Notice of Allowance, dated Aug. 4, 2017 issued in U.S. Appl. No. 14/167,908.
- U.S. Notice of Allowance, dated May 15, 2017, issued in U.S. Appl. No. 14/209,931.
- U.S. Notice of Allowance, dated Jun. 14, 2017, issued in U.S. Appl. No. 14/209,931.
- U.S. Office Action, dated May 25, 2018, issued in U.S. Appl. No. 15/062,045.
- U.S. Office Action, dated Jan. 19, 2017, issued in U.S. Appl. No. 14/808,361.
- U.S. Notice of Allowance, dated Apr. 28, 2017, issued in U.S. Appl. No. 14/808,361.
- U.S. Office Action, dated May 31, 2018, issued in U.S. Appl. No. 15/684,941.
- U.S. Office Action (Requirement for Restriction/Election), dated Jun. 8, 2017, issued in U.S. Appl. No. 14/858,527.
- U.S. Office Action dated Oct. 18, 2017 issued in U.S. Appl. No. 14/858,527.
- U.S. Notice of Allowance dated Apr. 6, 2018 issued in U.S. Appl. No. 14/858,527.
- U.S. Notice of Allowance dated Jun. 29, 2018 issued in U.S. Appl. No. 14/858,527.
- Databse Geneseq [Online] Jun. 15, 2007 (Jun. 15, 2007), "Medium chain-specific acyl-(ACP)-thioesterase CITEG1," retrieved from EBI accession No. GSP:AAW06703 Database accession No. AAW06703.
- Brazilian First Office Action dated Mar. 7, 2018 issued in Application No. BR 1120150179207.
- Chinese First Office Action dated Jun. 13, 2017 issued in CN 201480018889.4.
- Chinese Second Office Action dated Mar. 5, 2018 issued in CN 201480018889.4.
- European Second Office Action dated Jan. 4, 2018 issued in EP 14 706 996.7.
- Japanese First Office Action dated Mar. 29, 2018 issued in JP 2015-555436.
- Mexican Second Office Action dated Jun. 28, 2018 issued in MX/a/2015/009730.
- Malaysia Modified Substantive Examination Clear Report dated Sep. 28, 2018 issued in MY PI2015001876.
- Australian First Office Action dated Aug. 14, 2017 issued in AU 2014236763.
- Australian Second Office Action dated Jun. 12, 2018 issued in AU 2014236763.
- Australian Third Office Action dated Aug. 1, 2018 issued in AU 2014236763.
- European First Office Action dated Jul. 11, 2017 issued in EP 14769502.7.
- European Second Office Action [Examiner's Report] dated Mar. 5, 2018 issued in EP 14769502.7.
- European First Office Action dated Jan. 4, 2018 issued in EP 15747911.4.
- Mexican First Office Action dated Jan. 26, 2018 issued in MX MX/a/2015/011507.
- Mexican Second Office Action dated Jun. 15, 2018 issued in MX MX/a/2015/011507.
- Chinese First Office Action dated Apr. 23, 2018 issued in CN 201480020002.5.
- PCT International Preliminary Report on Patentability dated Mar. 30, 2017 issued in PCT/US2015/051042.
- European First Office Action dated Jun. 8, 2018 issued in EP 15775855.8.
- Brandt, et al. (1993) "Gametic Selection at Fatty Acid and Allozyme Marker Loci and Meiosis within *Cuphea viscosissima* x *Cuphea lanceolata* Populations," *Crop Science*, vol. 33, pp. 1138-1143.
- Jing et al., (2011) "Phylogenetic and experimental characterization of an acyl-ACP thioesterase family reveals significant diversity in enzymatic specificity and activity," *BMC Biochemistry*, Aug. 27, 2011, vol. 12.1, No. 44, pp. 1-16.
- Leonard et al., (1997) "Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids," *Plant Molecular Biology*, vol. 34, pp. 669-679.

* cited by examiner

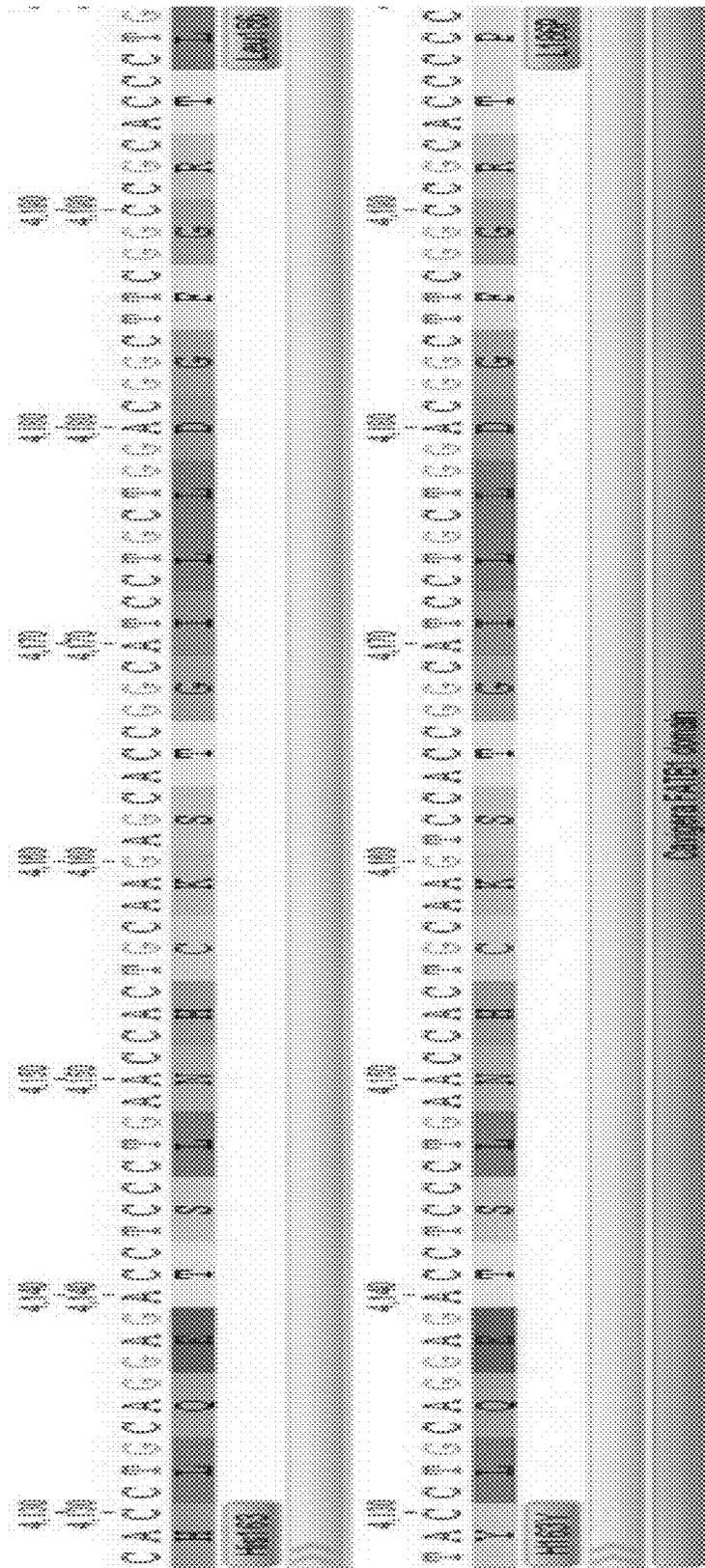


Fig. 1

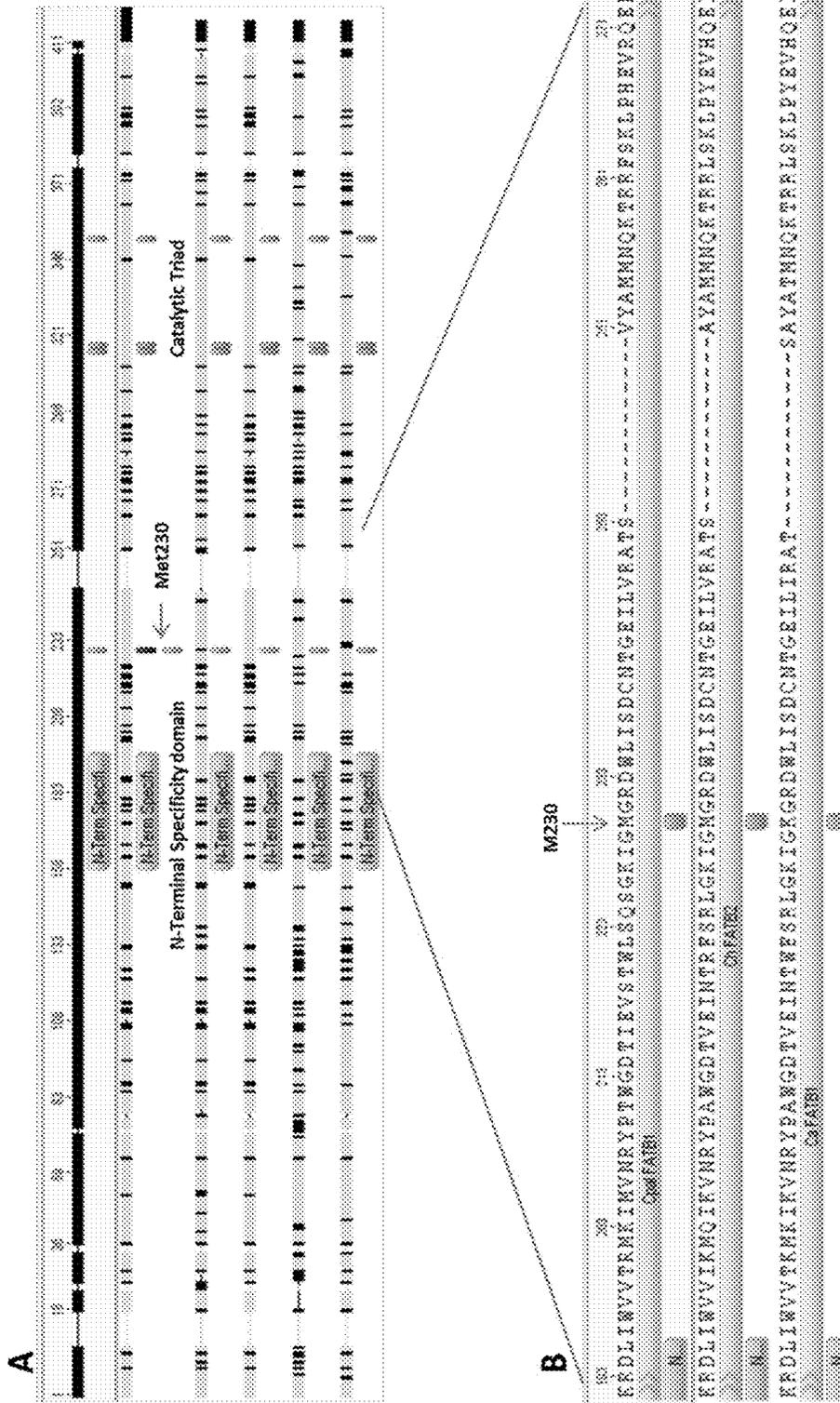


Fig. 2A-B

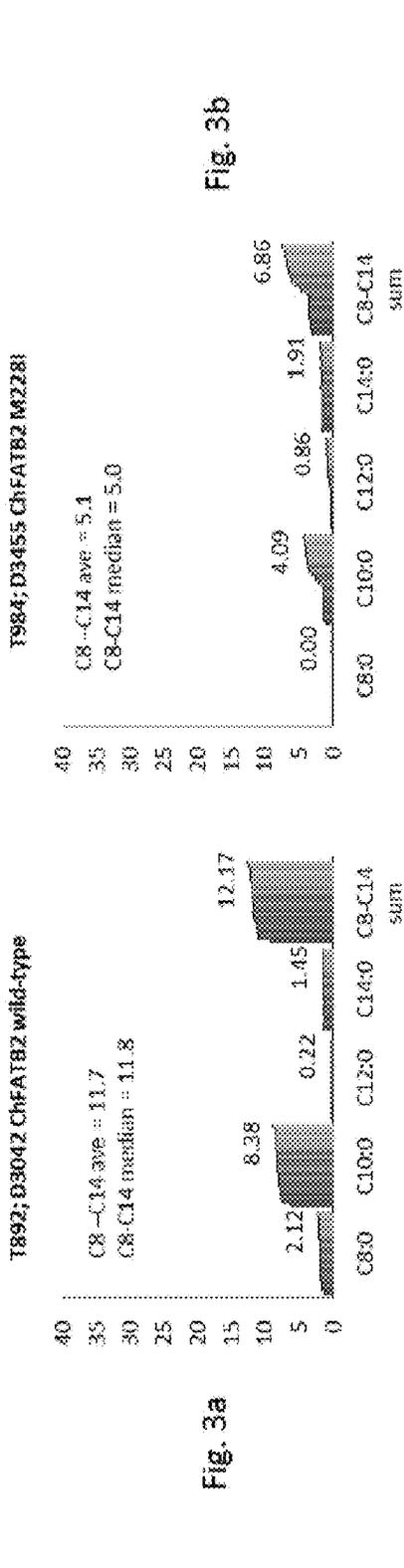
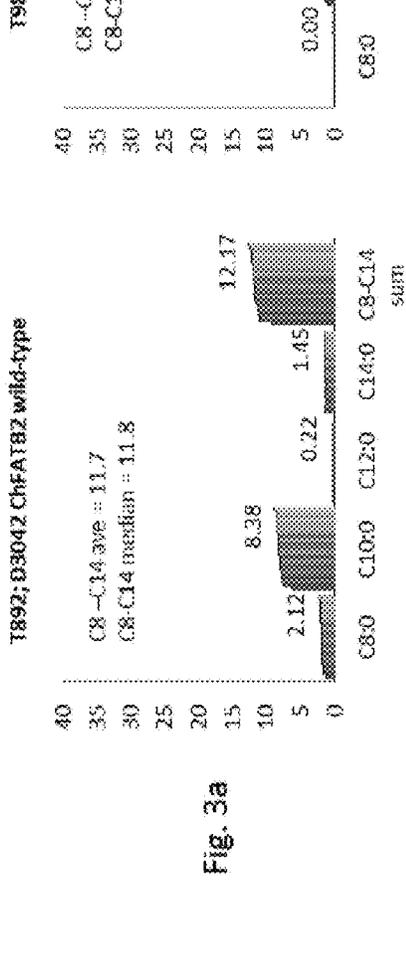
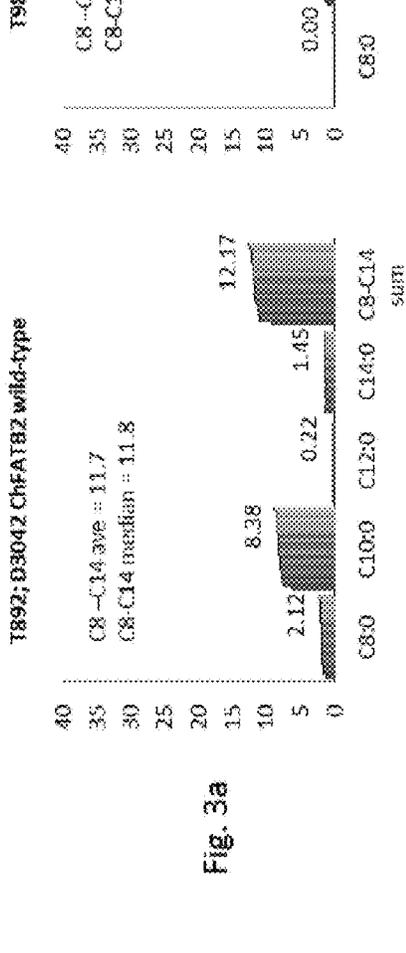
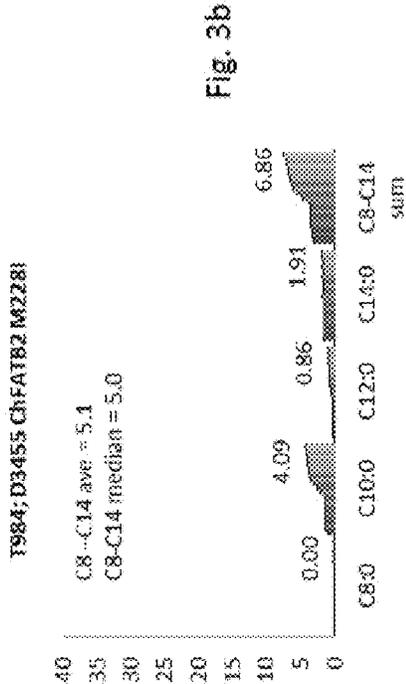
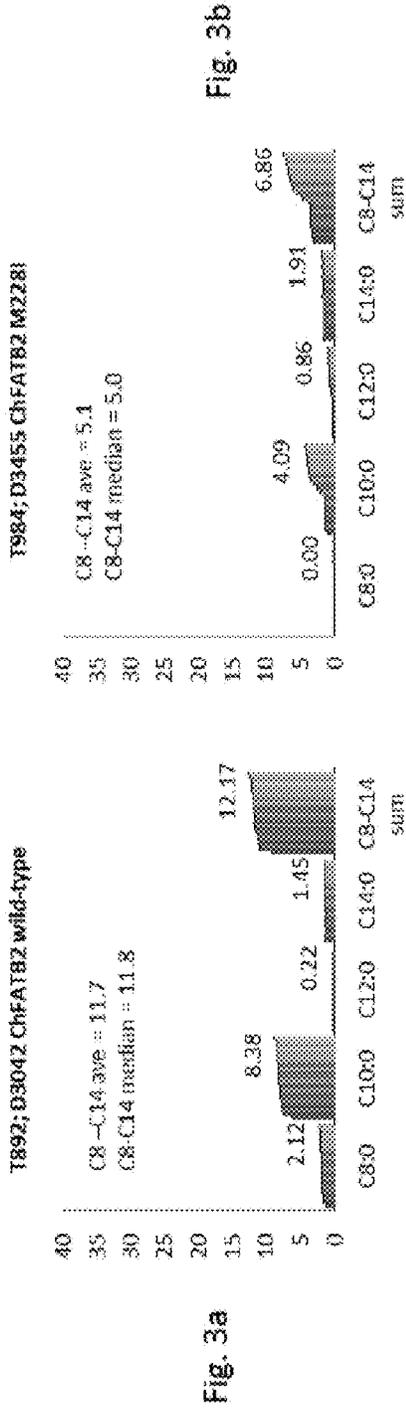


Fig. 3A-E

VARIANT THIOESTERASES AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 14/808,361, filed on Jul. 24, 2015, which claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 62/028,641, filed on Jul. 24, 2014, which are hereby incorporated herein by reference in their entireties for all purposes. This application is technologically related to the subject matter of PCT/US2014/013676, entitled "Variant Thioesterases and Methods of Use," and filed Jan. 29, 2014, which is hereby incorporated herein by reference in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Oct. 5, 2015, is named SOLAP027US_SL.txt and is 180,154 bytes in size.

FIELD

The present invention relates to variant acyl-ACP thioesterases and their use in oil-producing cells, e.g., to increase enzymatic activity toward certain acyl-ACP substrates and to promote increased production of oils with desired fatty acid profiles.

BACKGROUND

Today, fats and fatty acids primarily come from vegetable and animal sources, with the notable exception of commercial production of omega-3 fatty acids by fermentation of microbes for use in baby formula and nutritional supplements. Progress is being made however toward the commercial production of tailored oils using recombinant microalgae. See PCT Publications WO2008/151149, WO2010/06032, WO2011/150410, WO2011/150411, and international patent application PCT/US12/23696.

One method for producing a desired fatty acid profile in an oleaginous organism is to introduce an acyl-ACP thioesterase transgene; e.g., a transgene from a plant that produces a desired fatty acid.

By terminating fatty acid biosynthesis, the acyl-acyl carrier protein (ACP) thioesterase (TE) functionally determines the length and identity of the fatty acid end product (Salas et al., (2002) *Archives of Biochemistry and Biophysics* 403: 25-34). Based on amino acid sequence alignments, the plant TEs have been shown to cluster into two families, FatAs, which show marked preference for 18:1-ACP with minor activity towards 18:0- and 16:0-ACPs; and FatBs, which hydrolyze primarily saturated acyl-ACPs with chain lengths that vary between 8-16 carbons (Voelker, In Genetic Engineering Volume 18. Edited by: Setlow J K. New York, Plenum Press; 1996:111-133; Ginalski, et al., *Nucl Acids Res* (2003) 31:3291-3292; and Jones, et al., (1995) *Plant Cell* 7: 359-371). FatB TEs have a conserved hydrophobic 18-amino acid domain (Facciotti and Yuan (1998) *European Journal of Lipid Science and Technology* 100:167-172), and a conserved Asn-His-Cys catalytic triad in the C-terminal catalytic domain (Blatti, et al., *PLoS ONE* (2012) 7(9): e42949. doi:10.1371 and Mayer and Shanklin, *BMC Plant*

Biology (2007) 7:1-11). Mayer and Shanklin, *BMC Plant Biology* (2007) 7:1-11, identify a C-terminal conserved acyl-ACP thioesterase catalytic domain characterized by a C-terminal hot dog fold encompassing the Cys-His-Asn catalytic triad.

SUMMARY

Provided is a non-natural protein, an isolated gene encoding the non-natural protein, an expression cassette expressing the non-natural protein, or a host cell comprising the expression cassette. In some embodiments, the non-natural protein has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 1 and comprises Tyrosine (Y) or Phenylalanine (F) at the position corresponding to position 163 of SEQ ID NO: 1 and/or Proline (P), Lysine (K), or Alanine (A) at the position corresponding to position 186 of SEQ ID NO: 1. In some embodiments, the non-natural protein further comprises a Lysine (K) at the position corresponding to position 228 of SEQ ID NO: 1.

In a related aspect, provided is a method for producing a triglyceride oil. In varying embodiments, the methods comprise expressing, in a host cell, the protein of mentioned immediately above, or a protein comprising at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity one of SEQ ID NOs: 3-8 that has Y or F at the position corresponding to position 163 of SEQ ID NO: 1 and/or P, K, or A at the position corresponding to position 186 of SEQ ID NO: 1. In some embodiments, the non-natural protein further comprises K at the position corresponding to position 228 of SEQ ID NO: 1. The method further includes cultivating the host cell and isolating the oil.

In another aspect, provided is a method for increasing the C8 and/or C10 fatty acids in a fatty acid profile of an oil produced by an optionally oleaginous host cell. The method includes, providing a parent gene encoding a FATB enzyme, mutating the gene to so as to have Y or F at the position corresponding to position 163 of SEQ ID NO: 1 and/or P, K, or A at the position corresponding to position 186 of SEQ ID NO: 1. In some embodiments, the non-natural protein further comprises K at the position corresponding to position 228 of SEQ ID NO: 1. In varying embodiments, the method further includes expressing the mutated gene in the host cell and producing the oil. The fatty acid profile of the oil is thereby increased in C8 and/or C10 fatty acids relative to the parent gene. Optionally, the gene encoding the FATB enzyme encodes a protein with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 1, 13 or 14.

In an embodiment, provided is a non-natural protein, an isolated gene encoding the non-natural protein, an expression cassette expressing the non-natural protein, or a host cell comprising the expression cassette. In varying embodiments, the non-natural protein has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 13 and A or K at the position corresponding to position 230 of SEQ ID NO: 13. A method for producing an oil includes expressing, in a host cell, the non-natural proteins described herein, cultivating the cell, and isolating the oil.

In another aspect, provided is a non-natural protein, an isolated gene encoding the non-natural protein, an expression cassette expressing the non-natural protein, or a host cell comprising the expression cassette. In some embodiments, the non-natural protein has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%

identity to SEQ ID NO: 45 and comprises A, T or V at the position corresponding to position 74 of SEQ ID NO: 45 (G96 of wild-type Gm FATA) and/or F, K or S at the position corresponding to position 69 of SEQ ID NO: 45 (L91 of wild-type Gm FATA), and/or F, A, K or V at the position corresponding to position 134 of SEQ ID NO: 45 (T156 of wild-type Gm FATA). In some embodiments, the non-natural protein further comprises A or V at the position corresponding to position 89 of SEQ ID NO: 45 (S111 of wild-type Gm FATA) and/or A at the position corresponding to position 171 of SEQ ID NO: 45 (V193 of wild-type Gm FATA), and/or A or V at the position corresponding to position 86 of SEQ ID NO: 45 (G108 of wild-type Gm FATA).

In a further aspect, provided is a method for producing a triglyceride oil. In various embodiments, the method comprises expressing, in a host cell, the protein of claim 7 or claim 8, or a protein comprising at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to one of SEQ ID NOs: 45 and 15-29 and comprises A, T or V at the position corresponding to position 74 of SEQ ID NO: 45 (G96 of wild-type Gm FATA) and/or F, K or S at the position corresponding to position 69 of SEQ ID NO: 45 (L91 of wild-type Gm FATA), and/or F, A, K or V at the position corresponding to position 134 of SEQ ID NO: 45 (G156 of wild-type Gm FATA); cultivating the host cell; and isolating the oil. In some embodiments, the protein further comprises A or V at the position corresponding to position 89 of SEQ ID NO: 45 (S111 of wild-type Gm FATA) and/or A at the position corresponding to position 171 of SEQ ID NO: 45 (V193 of wild-type Gm FATA), and/or A or V at the position corresponding to position 86 of SEQ ID NO: 45 (G108 of wild-type Gm FATA).

In another aspect, provided is a method for increasing the C18:0 fatty acids in a fatty acid profile of an oil produced by an optionally oleaginous host cell. In some embodiments, the method further comprises providing a parent gene encoding a FATB enzyme, mutating the gene to so as to have A, T or V at the position corresponding to position 74 of SEQ ID NO: 45 (G96 of wild-type Gm FATA) and/or F, K or S at the position corresponding to position 69 of SEQ ID NO: 45 (L91 of wild-type Gm FATA), and/or F, A, K or V at the position corresponding to position 134 of SEQ ID NO: 45 (T156 of wild-type Gm FATA); expressing the mutated gene in the host cell; and producing the oil, whereby the fatty acid profile of the oil is increased in C18:0 fatty acids relative to the parent gene. In various embodiments, the method entails further mutating the gene to so as to have A or V at the position corresponding to position 89 of SEQ ID NO: 45 (S111 of wild-type Gm FATA) and/or A at the position corresponding to position 171 of SEQ ID NO: 45 (V193 of wild-type Gm FATA), and/or A or V at the position corresponding to position 86 of SEQ ID NO: 45 (G108 of wild-type Gm FATA). In some embodiments, the gene encoding the FATB enzyme encodes a protein with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to one of SEQ ID NOs: 45 and 15-29.

Definitions

An “acyl-ACP thioesterase” or “acyl-ACP TE” interchangeably refer to an enzyme that catalyzes the cleavage of a fatty acid from an acyl carrier protein (ACP) during lipid synthesis. Acyl-acyl carrier protein (ACP) thioesterases (TEs) hydrolyze acyl-ACP thioester bonds, releasing free fatty acids and ACP.

The term “acyl-ACP preferring TE” refers to the fatty acyl-ACP substrate specificity of a TE. An acyl-ACP preferring TE preferentially liberates a particular fatty acid from an acyl-ACP substrate. For example, the acyl-ACP preferring TE can preferentially liberate a given fatty acid over all other fatty acids in the set of C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, and C18:2 fatty acids. The preference of the acyl-ACP preferring TE can be detected as a higher V_{max} (or a higher k_{cat} , or a higher V/K) in comparison to other non-preferred fatty acid-ACP substrates. The preference can be inferred from changes in fatty acid profile of a cell genetically engineered to overexpress the acyl-ACP preferring TE relative to a control cell that does not overexpress the acyl-ACP preferring TE.

Numbering of a given amino acid polymer or nucleic acid polymer “corresponds to” or is “relative to” the numbering of a selected amino acid polymer or nucleic acid polymer when the position of any given polymer component (e.g., amino acid, nucleotide, also referred to generically as a “residue”) is designated by reference to the same or to an equivalent position (e.g., based on an optimal alignment or a consensus sequence) in the selected amino acid or nucleic acid polymer, rather than by the actual numerical position of the component in the given polymer.

A “variant” is a polypeptide comprising a sequence which differs in one or more amino acid position(s) from that of a parent polypeptide sequence (e.g., by substitution, deletion, or insertion). A variant may comprise a sequence which differs from the parent polypeptides sequence in up to 40% of the total number of residues of the parent polypeptide sequence, such as in up to 40%, 35%, 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3% 2% or 1% of the total number of residues of the parent polypeptide sequence. For example, a variant of a 400 amino acid polypeptide sequence comprises a sequence which differs in up to 40% of the total number of residues of the parent polypeptide sequence, that is, in up to 160 amino acid positions within the 400 amino acid polypeptide sequence (such as in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 78, 79, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, or 160 amino acid positions within the reference sequence.

“Naturally occurring” as applied to a composition that can be found in nature as distinct from being artificially produced by man. For example, a polypeptide or polynucleotide that is present in an organism (including viruses, bacteria, protozoa, insects, plants or mammalian tissue) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring. “Non-naturally occurring” (also termed “synthetic” or “artificial”) as applied to an object means that the object is not naturally-occurring—i.e., the object cannot be found in nature as distinct from being artificially produced by man.

A “cell oil” or “cell fat” shall mean a predominantly triglyceride oil obtained from an organism, where the oil has not undergone blending with another natural or synthetic oil, or fractionation so as to substantially alter the fatty acid profile of the triglyceride. In connection with an oil comprising triglycerides of a particular regiospecificity, the cell oil or cell fat has not been subjected to interesterification or other synthetic process to obtain that regiospecific triglyceride profile, rather the regiospecificity is produced naturally, by a cell or population of cells. For a cell oil or cell fat

produced by a cell, the sterol profile of oil is generally determined by the sterols produced by the cell, not by artificial reconstitution of the oil by adding sterols in order to mimic the cell oil. In connection with a cell oil or cell fat, and as used generally throughout the present disclosure, the terms oil and fat are used interchangeably, except where otherwise noted. Thus, an "oil" or a "fat" can be liquid, solid, or partially solid at room temperature, depending on the makeup of the substance and other conditions. Here, the term "fractionation" means removing material from the oil in a way that changes its fatty acid profile relative to the profile produced by the organism, however accomplished. The terms "cell oil" and "cell fat" encompass such oils obtained from an organism, where the oil has undergone minimal processing, including refining, bleaching and/or degumming, which does not substantially change its triglyceride profile. A cell oil can also be a "noninteresterified cell oil", which means that the cell oil has not undergone a process in which fatty acids have been redistributed in their acyl linkages to glycerol and remain essentially in the same configuration as when recovered from the organism.

A "fatty acid profile" is the distribution of fatty acyl groups in the triglycerides of the oil without reference to attachment to a glycerol backbone. Fatty acid profiles are typically determined by conversion to a fatty acid methyl ester (FAME), followed by gas chromatography (GC) analysis with flame ionization detection (FID). The fatty acid profile can be expressed as one or more percent of a fatty acid in the total fatty acid signal determined from the area under the curve for that fatty acid. FAME-GC-FID measurement approximate weight percentages of the fatty acids.

"Microalgae" are microbial organisms that contain a chloroplast or plastid, and optionally that is capable of performing photosynthesis, or a prokaryotic microbial organism capable of performing photosynthesis. Microalgae include obligate photoautotrophs, which cannot metabolize a fixed carbon source as energy, as well as heterotrophs, which can live solely off of a fixed carbon source. Microalgae include unicellular organisms that separate from sister cells shortly after cell division, such as *Chlamydomonas*, as well as microbes such as, for example, *Volvox*, which is a simple multicellular photosynthetic microbe of two distinct cell types. Microalgae include eukaryotic Chlorophyceae such as *Chlorella*, *Dunaliella*, and *Prototheca*. Microalgae also include other microbial photosynthetic organisms that exhibit cell-cell adhesion, such as *Agmenellum*, *Anabaena*, and *Pyrobotrys*. Microalgae also include obligate heterotrophic microorganisms that have lost the ability to perform photosynthesis, such as certain dinoflagellate algae species and species of the genus *Prototheca*.

An "oleaginous" cell is a non-human cell capable of producing at least 20% lipid by dry cell weight, naturally or through recombinant or classical strain improvement. An "oleaginous microbe" or "oleaginous microorganism" is a microbe, including a microalga that is oleaginous.

As used with respect to polypeptides or polynucleotides, the term "isolated" refers to a polypeptide or polynucleotide that has been separated from at least one other component that is typically present with the polypeptide or polynucleotide. Thus, a naturally occurring polypeptide is isolated if it has been purified away from at least one other component that occurs naturally with the polypeptide or polynucleotide. A recombinant polypeptide or polynucleotide is isolated if it has been purified away from at least one other component present when the polypeptide or polynucleotide is produced.

The terms "polypeptide" and "protein" are used interchangeably herein to refer a polymer of amino acids, and

unless otherwise limited, include atypical amino acids that can function in a similar manner to naturally occurring amino acids.

The term "sequence", as used in connection with a polypeptide or nucleic acid polymer refers to the order of monomers making up the polymer or the sub-polymer or fragment having that sequence.

A "subsequence" of an amino acid or nucleotide sequence is a portion of a larger sequence or the peptide or nucleic acid sub-polymer or fragment characterized by the portion of the larger sequence.

The terms "identical" or "percent identity," in the context of two or more amino acid or nucleotide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection.

For sequence comparison to determine percent nucleotide or amino acid identity, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. Optimal alignment of sequences for comparison can be conducted using BLAST set to default parameters.

As used with reference to polypeptides, the term "wild-type" refers to any polypeptide having an amino acid sequence present in a polypeptide from a naturally occurring organism, regardless of the source of the molecule; i.e., the term "wild-type" refers to sequence characteristics, regardless of whether the molecule is purified from a natural source; expressed recombinantly, followed by purification; or synthesized.

The term "mutation" shall mean a change in a protein, polypeptide, or peptide sequence or subsequence produced by altering one or more nucleotides in a nucleotide coding for the protein, polypeptide, or peptide, however the alteration is obtained. For example, a mutation can be produced randomly, by PCR mutation, by synthesis of entire gene, or any other method.

The term "vector" is used herein to describe a DNA construct containing a polynucleotide. Such a vector can be propagated stably or transiently in a host cell. The vector can, for example, be a plasmid, a viral vector, or simply a potential genomic insert. Once introduced into a suitable host, the vector may replicate and function independently of the host genome, or may, in some instances, integrate into the host genome.

As used herein, the terms "expression vector" or "expression construct" or "expression cassette" refer to a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a host cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment. An "expression cassette" includes a coding nucleic acid (CDS) to be transcribed operably linked to a promoter and a 3'UTR. Optionally, and in the Examples below, the promoter of an expression cassette is a heterologous promoter.

"Exogenous gene" refers to a nucleic acid transformed into a cell. The exogenous gene may be from a different species (and so heterologous), or from the same species (and

so homologous) relative to the cell being transformed. In the case of a homologous gene, it occupies a different location in the genome of the cell relative to the endogenous copy of the gene. The exogenous gene may be present in more than one copy in the cell. The exogenous gene may be maintained in a cell as an insertion into the genome or as an episomal molecule.

An “inducible promoter” is one that mediates transcription of an operably linked gene in response to a particular stimulus.

As used herein, the phrase “in operable linkage” refers to a functional linkage between two sequences, such a control sequence (typically a promoter) and the linked sequence. A promoter is in operable linkage with an exogenous gene if it can mediate transcription of the gene.

A “promoter” is defined as an array of nucleic acid control sequences that direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription.

As used herein, the term “recombinant” when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of an exogenous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, over-expressed, under-expressed or not expressed at all. “Recombinant nucleic acid” as used herein refers to nucleic acid molecules that are initially synthesized through the use of laboratory methods, thereby creating nucleic acid sequences that are not normally found in nature. By using laboratory methods, recombinant nucleic acid molecules in operable linkage with different sequences (e.g., promoter, targeting sequence, etc.) is achieved. Thus an isolated nucleic acid, in a linear form, or an expression vector formed in vitro by ligating DNA molecules that are not normally joined, are both considered recombinant for the purposes of this invention. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e., using the in vivo cellular machinery of the host cell rather than in vitro manipulations; however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purposes herein. Similarly, a “recombinant protein” is a protein made using recombinant techniques, i.e., through the expression of a recombinant nucleic acid as depicted above.

A “transit peptide” is an amino acid sequence that directs the trafficking of a polypeptide fused to the signal sequence. In connection with plastidic cells expressing the polypeptide, the transit peptide may direct trafficking of the polypeptide to the plastid (i.e., a plastid targeting peptide).

The term “polynucleotide” refers to a deoxyribonucleotide or ribonucleotide polymer, and unless otherwise limited, includes known analogs of natural nucleotides that can function in a similar manner to naturally occurring nucleotides. The term “polynucleotide” refers any form of DNA or RNA, including, for example, genomic DNA; complementary DNA (cDNA), which is a DNA representation of mRNA, usually obtained by reverse transcription of mes-

senger RNA (mRNA) or amplification; DNA molecules produced synthetically or by amplification; and mRNA. The term “polynucleotide” encompasses double-stranded nucleic acid molecules, as well as single-stranded molecules. In double-stranded polynucleotides, the polynucleotide strands need not be coextensive (i.e., a double-stranded polynucleotide need not be double-stranded along the entire length of both strands).

The term “host cell” refers to a cell capable of maintaining a vector either transiently or stably. Host cells include, without limitation, bacterial cells, yeast cells, insect cells, algal cells (e.g., microalgal cells), plant cells and mammalian cells. Other host cells known in the art, or which become known, are also suitable for use in the invention.

As used herein, the term “complementary” refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a given position of a nucleic acid molecule is capable of hybridizing with a nucleotide of another nucleic acid molecule, then the two nucleic acid molecules are considered to be complementary to one another at that position. The term “substantially complementary” describes sequences that are sufficiently complementary to one another to allow for specific hybridization under stringent hybridization conditions. In various embodiments, the variant genes encoding variant FATB genes disclosed below can be replaced with a substantially complementary gene having suitable activity.

The phrase “stringent hybridization conditions” generally refers to a temperature about 5° C. lower than the melting temperature (T_m) for a specific sequence at a defined ionic strength and pH. Exemplary stringent conditions suitable for achieving specific hybridization of most sequences are a temperature of at least about 60° C. and a salt concentration of about 0.2 molar at pH 7.0.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates a sequence alignment of the *Cuphea hookeriana* FATB2 (SEQ ID NOS 69 and 70, respectively, in order of appearance) versus the *Cuphea avigera* FATB1 (SEQ ID NOS 71 and 72, respectively, in order of appearance) illustrates the two amino acid differences between these thioesterases within their N-terminal specificity domain.

FIGS. 2A-B illustrate (A) a sequence alignment of FATB thioesterases isolated from *Cuphea* genomes. The position of the conserved Methionine relative to the Catalytic Triad (Cys, His, and Asn) and N-terminal Specificity domain is highlighted; and (B) a sequence comparison of the Cpal FATB1, Ch FATB2 and Ca FATB1 surrounding the highlighted methionine (SEQ ID NOS 73-75, respectively, in order of appearance). The Ca FATB1 is unique due to the presence of a lysine instead of the methionine.

FIGS. 3A-E illustrate histograms of C8-C14 fatty acid profiles of microalgal oil with mean and median values for multiple transformants of wild type and position 228 variant *Cuphea hookeriana* FATB2 (ChFATB2), *Cuphea avigera* FATB1 (CaFATB1) that depart from predictions based on prior data from an *E. coli* model.

DETAILED DESCRIPTION

Introduction

In illustrative embodiments, variant FATB acyl-ACP thioesterases described herein allow for control over acyl-ACP thioesterase substrate specificity. As a result, host cells

expressing the acyl-ACP thioesterases produce oils with altered fatty acid profiles. In certain embodiments host cells expressing the variant acyl-ACP thioesterases produce triglyceride-rich cell oils with fatty acid profiles characterized by elevated mid chain fatty acids such as C8:0, C10:0, C12:0, and C14:0 fatty acids. A specific embodiment includes providing a FATB acyl-ACP thioesterase gene, mutating the gene so as to alter the amino acids in the gene product at the positions corresponding to H163 and/or L186 of the reference *Cuphea hookeriana* FATB2 gene (SEQ ID NO: 1). Optionally, the H163 and/or L186 mutant is combined with a mutation at M228.

As described in more detail in Example 1, by expressing such variant FATB2 genes, stably integrated in the nucleus of oleaginous plastidic cells, we produced strains that exceeded wildtype ChFATB2 expressing control strains in terms of C8:0, C8:10 or the sum of C8:0 and C10:0 production, including strains that produced oils with fatty acid profiles where the C8 and C10 production exceed 9, 11, 14, or 18% of the profile. In the latter case, the C8+C10 (i.e., the sum of C8:0 and C10:0 production in the fatty acid profile as determined by FAME-GC with FID detection) level was more than doubled relative to the approximately 8% C8+C10 of the wildtype ChFATb2 strain. Specific variants with improved C8+C10 production include those with P, K, or A at the 186 position; Y or F at the 163 position, or combinations thereof such as 186P/163Y, 186P/163F, 186K/163Y, 186K/163F, 186A/163Y or 186A/163F. Of the double mutants, we found that the H163Y/L186P variant produced an oil having particularly high concentrations of C8+C10. Using single or double variants, the C8:0 fatty acid profile percentages can be increased by 50, 60, 70, 80, 100% or more relative to a control strain expressing wildtype ChFATB2; e.g. to more than 2, 2.5, 3, or 3.5% of the fatty acid profile vs. 1.5% for the control (see Example 1).

The double mutants listed above can also be combined with a third mutation corresponding to 230 of *Cuphea palustris* FATB1. For many FATB genes such as *Cuphea hookeriana* FATB2 and *Cuphea avigera* FATB1, this residue corresponds to residue 228. For example, an M228K mutation in *Cuphea hookeriana* FATB2 expressed in an oleaginous eukaryotic microalga increased the C8/C10 ratio in the fatty acid profile of the oil from about 0.25 to about 1.0. Mutations at this position to Iso, Val, Phe, and Leu, Ala, or Thr in combination with the single or double mutants at positions 186 and 163 discussed above, can also be advantageous.

Although *Cuphea hookeriana* FATB2 was used as a model system, the methods of making the above-discussed mutations, methods of expressing these in an oleaginous cell, and methods of producing oil with these variants can be applied to any acyl-ACP thioesterase gene, including those having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:1, or the fragment of SEQ ID NO: 1 lacking the transit peptide.

Although these variant genes were discovered using a eukaryotic microalgal expression system, the genes are more generally useful in ways that are known in the art, including their expression in higher plants to produced altered triglyceride oils. When incorporated into an oleaginous cell (e.g., of an oilseed plant, algae (e.g., microalgae)) the variant thioesterases can alter the fatty acid profiles of the cell to produce novel or more economical high-value commercial products.

The single, double or triple mutants can be used to produce an oil with a high ratio of C8:0 to C10:0 fatty acids.

For example, the C8/10 ratio can be equal to or greater than 0.3, 0.5, 0.7, 0.9, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, or 3.0.

The embodiments also encompass the residual biomass from such cells after oil extraction, oleochemicals, fuels and food products made from the oils and methods of cultivating the cells. In varying embodiments, the cells are microalgal cells, including heterotrophic or obligate heterotrophic cells, and cells classified as Chlorophyta, Trebouxiophyceae, Chlorellales, Chlorellaceae, or Chlorophyceae. The cells can also be plant cells or cells of macroalgae. Host cells having a type II fatty acid synthesis pathway are preferred. Although the examples given below use the Trebouxiophyte *Prototheca moriformis* as a host cell, the genes, constructs and methods disclosed may also find use in oilseed crops. Methods for introducing these genes into such crops such as soybean, corn, rapeseed, safflower, sunflower and others are known in the art; see, for example, U.S. Pat. Nos. 6,331,664, 5,512,482, 5,455,167, 5,667,997. Examples of oleochemicals include surfactants and solvents made from fatty acids or oils.

Accordingly, in an embodiment, provided is a non-natural protein, an isolated gene encoding the non-natural protein, an expression cassette expressing the non-natural protein, or a host cell comprising the expression cassette, wherein the non-natural protein has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 1 and comprises Y or F at the position corresponding to position 163 of SEQ ID NO: 1 and/or P, K, or A at the position corresponding to position 186 of SEQ ID NO: 1, and optionally K at the position corresponding to position 228 of SEQ ID NO: 1.

In a related embodiment, there is a method for producing a triglyceride oil. The method includes expressing, in a host cell, the protein of mentioned immediately above, or a protein comprising at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity one of SEQ ID NOs: 3-8 that has Y or F at the position corresponding to position 163 of SEQ ID NO: 1 and/or P, K, or A at the position corresponding to position 186 of SEQ ID NO: 1, and optionally K at the position corresponding to position 228 of SEQ ID NO: 1. The method further includes cultivating the host cell and isolating the oil.

In another embodiment, provided is a method for increasing the C8 and/or C10 fatty acids in a fatty acid profile of an oil produced by an optionally oleaginous host cell. The method includes, providing a parent gene encoding a FATB enzyme, mutating the gene to so as to have Y or F at the position corresponding to position 163 of SEQ ID NO: 1 and/or P, K, or A at the position corresponding to position 186 of SEQ ID NO: 1, and optionally K at the position corresponding to position 228 of SEQ ID NO: 1. The method further includes expressing the mutated gene in the host cell and producing the oil. The fatty acid profile of the oil is thereby increased in C8 and/or C10 fatty acids relative to the parent gene. Optionally, the gene encoding the FATB enzyme encodes a protein with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 1, 13 or 14.

As detailed in Example 3, compared to prior art work in *E. Coli*, the discovery of the advantage of using Ala, or Thr at position 230 of Cpal FATB1 (SEQ ID NO: 13) of in terms of C8+C10 production and/or increased C8/C10 ratio, is new and unexpected. These novel mutations are useful alone, in combination with a mutation at position 163 including the C8-favoring mutations disclosed herein, in combination with a mutation at position 186 including the C8-favoring

mutations disclosed herein, or in combination with a double mutation at positions 163 and 186 including the C8-favoring mutations disclosed herein. Accordingly, in an embodiment, there is a non-natural protein, an isolated gene encoding the non-natural protein, an expression cassette expressing the non-natural protein, or a host cell comprising the expression cassette, wherein the non-natural protein has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 13 and A or K at the position corresponding to position 230 of SEQ ID NO: 13. A method for producing an oil includes expressing, in a host cell, the non-natural proteins described herein, cultivating the cell, and isolating the oil.

Variant Acyl-ACP Thioesterases

The variant TEs can be used in genetic constructs and genetically engineered oleaginous cells (e.g., plants, algae, microalgae) with one or more exogenous genes to produce fatty acids, acylglycerides, or derivatives thereof. For example, microalgae or oilseed crops that would naturally, or through genetic modification, produce high levels of lipids can be engineered (or further engineered) to express an exogenous variant fatty acyl-ACP thioesterase, which can facilitate the cleavage of fatty acids from acyl carrier protein (ACP) during fatty acid synthesis. The fatty acids synthesized may be incorporated into acyl glycerides including triacylglycerides (TAGs, triglycerides). The TAGs can be recovered or, through further enzymatic processing within the cell, or in vitro, yield other useful compounds.

In an embodiment, the variant fatty acyl-ACP thioesterases are designed based on the desired specificity for a growing (during fatty acid synthesis) fatty acyl group having a particular carbon chain length. A specificity domain is selected based on its preference for a particular fatty acyl ACP substrate and/or for its ability to influence, increase and/or promote the production of fatty acids of a desired carbon chain length. Generally, the variant fatty acyl-ACP thioesterases have preferential substrate specificity for mid-chain ACP-fatty acyl substrates (e.g., to liberate C8, C10, C12, and/or C14 fatty acids). In varying embodiments, the specificity domain in the N-terminus of the acyl-ACP thioesterase is heterologous (e.g., due to point mutations and/or domain swapping) to the C-terminal catalytic domain. In certain embodiments, the fatty acid chain length substrate specificity and/or preference of the specificity domain and the catalytic domain is the same or within 1-2 carbons. For example, in varying embodiments, the variant acyl-acyl carrier protein (ACP) thioesterase (TE) comprises:

Codon-Optimization for Expression

DNA encoding a polypeptide to be expressed in a microorganism, e.g., a variant acyl-ACP thioesterase and selectable marker can be codon-optimized cDNA. Methods of recoding genes for expression in microalgae are described in U.S. Pat. No. 7,135,290. Additional information for codon optimization is available, e.g., at the Codon Usage Database at kazusa.or.jp/codon/. The table for *Prototheca* preferred codon usage is also provided in U.S. Patent Publ. No. 2012/0283460, Table 1 of which is hereby incorporated herein by reference.

Expression and Targeting to Plastids

Proteins expressed in the nuclear genome of *Prototheca* can be targeted to the plastid using plastid targeting signals. Plastid targeting sequences endogenous to *Chlorella* are known, such as genes in the *Chlorella* nuclear genome that encode proteins that are targeted to the plastid; see for example GenBank Accession numbers AY646197 and AF499684, and in one embodiment, such control sequences

are used in the vectors described herein, e.g., to target expression of a protein to a *Prototheca* plastid.

The Examples below describe the use of algal plastid targeting sequences to target heterologous proteins to the correct compartment in the host cell. cDNA libraries were made using *Prototheca moriformis* and *Chlorella protothecoides* cells and are described in the Examples of U.S. Patent Publ. No. 2012/0283460 and in PCT Application No. PCT/US2009/066142. Amino acid sequences of the algal plastid targeting sequences identified from the cDNA libraries useful plastid targeting of recombinantly expressed variant acyl-ACP thioesterases are provided in U.S. Patent Publ. No. 2012/0283460 and herein. In varying embodiments, the plastid transit peptide comprises an amino acid sequence selected from the group consisting of

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MATASTFSAFNARCGDLRRSAGSGPRRRPARPLPVRGRA,
SGPRRRPARPLPVR, SGPRRRPARPLPVRAAIASEVPVATTSPR,
RPARPLPVRGRA, RPARPLPVRAAIASEVPVATTSPR,
RCGDLRRSAGSGPRRRPARPLPVRGRA,
RCGDLRRSAGSGPRRRPARPLPVRAAIASEVPVATTSPR,
PARPLPVR, PARPLPVRAAIASEVPVATTSPR,
RRPARPLPVR,
and
RRPARPLPVRAAIASEVPVATTSPR.

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Where novel FATB variants are disclosed here, it will be understood that a variety of heterologous plastid transit peptides can be used. In other words, the non-targeting peptide domain is more highly conserved. Accordingly, embodiments described herein feature the novel FATB enzymatic domain with or without a plastid targeting sequence. For example, where a percent identity to a novel FATB gene is given herein, the same identity can be applied (where specified) to the same sequence absent the targeting peptide. A substitute targeting peptide can optionally be used in connection with such a sequence.

Host Cells—Oil- or Lipid-Producing Microorganisms

Any species of organism that produces suitable lipid and/or hydrocarbon can be used, although microorganisms that naturally produce high levels of suitable lipid and/or hydrocarbon are preferred. Production of hydrocarbons by microorganisms is reviewed by Metzger et al. Appl Microbiol Biotechnol (2005) 66: 486-496 and A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae, NREUTP-580-24190, John Sheehan, Terri Dunahay, John Benemann and Paul Roessler (1998).

Considerations for the selection of microorganisms include, in addition to production of suitable lipids or hydrocarbons for production of oils, fuels, and oleochemicals: (1) high lipid content as a percentage of cell weight; (2) ease of growth; (3) ease of genetic engineering; and (4) ease of biomass processing. In particular embodiments, the wild-type or genetically engineered microorganism yields cells that are at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, or at least 70% or more lipid. Preferred organisms grow heterotrophically (on sugars in the absence of light) or can be engineered to do so using, for example, methods disclosed herein. The ease of transformation and availability of selectable markers and promoters, constitutive or inducible, that are functional in the microorganism affect the ease of genetic engineering. Processing

considerations can include, for example, the availability of effective means for lysing the cells.

A. Algae

In one embodiment, the microorganism is a microalgae. Nonlimiting examples of microalgae that can be used for expression of variant acyl-ACP thioesterases include, e.g., *Achnanthes orientalis*, *Agmenellum*, *Amphiprora hyaline*, *Amphora coffeiformis*, *Amphora coffeiformis lineae*, *Amphora coffeiformis punctata*, *Amphora coffeiformis taylori*, *Amphora coffeiformis tenuis*, *Amphora delicatissima*, *Amphora delicatissima capitata*, *Amphora* sp., *Anabaena*, *Ankistrodesmus*, *Ankistrodesmus falcatus*, *Boekelovia hooglandii*, *Borodinella* sp., *Botryococcus braunii*, *Botryococcus sudeticus*, *Bracteococcus minor*, *Bracteococcus medionucleatus*, *Carteria*, *Chaetoceros gracilis*, *Chaetoceros muelleri*, *Chaetoceros muelleri subsalsum*, *Chaetoceros* sp., *Chlorella anitrata*, *Chlorella Antarctica*, *Chlorella aureoviridis*, *Chlorella candida*, *Chlorella capsulate*, *Chlorella desiccata*, *Chlorella ellipsoidea*, *Chlorella emersonii*, *Chlorella fusca*, *Chlorella fusca* var. *vacuolata*, *Chlorella glucotropha*, *Chlorella infusionum*, *Chlorella infusionum* var. *actophila*, *Chlorella infusionum* var. *auxenophila*, *Chlorella kessleri*, *Chlorella lobophora* (strain SAG 37.88), *Chlorella luteoviridis*, *Chlorella luteoviridis* var. *aureoviridis*, *Chlorella luteoviridis* var. *lutescens*, *Chlorella miniata*, *Chlorella minutissima*, *Chlorella mutabilis*, *Chlorella nocturna*, *Chlorella ovalis*, *Chlorella parva*, *Chlorella photophila*, *Chlorella pringsheimii*, *Chlorella protothecoides* (including any of UTEX strains 1806, 411, 264, 256, 255, 250, 249, 31, 29, 25), *Chlorella protothecoides* var. *acidicola*, *Chlorella regularis*, *Chlorella regularis* var. *minima*, *Chlorella regularis* var. *umbricata*, *Chlorella reisigii*, *Chlorella saccharophila*, *Chlorella saccharophila* var. *ellipsoidea*, *Chlorella salina*, *Chlorella simplex*, *Chlorella sorokiniana*, *Chlorella* sp., *Chlorella sphaerica*, *Chlorella stigmatophora*, *Chlorella vanniellii*, *Chlorella vulgaris*, *Chlorella vulgaris* f. *tertia*, *Chlorella vulgaris* var. *autotrophica*, *Chlorella vulgaris* var. *viridis*, *Chlorella vulgaris* var. *vulgaris*, *Chlorella vulgaris* var. *vulgaris* f. *tertia*, *Chlorella vulgaris* var. *vulgaris* f. *viridis*, *Chlorella xanthella*, *Chlorella zofingiensis*, *Chlorella trebouxioides*, *Chlorella vulgaris*, *Chlorococcum infusionum*, *Chlorococcum* sp., *Chlorogonium*, *Chroomonas* sp., *Chryso-sphaera* sp., *Cricosphaera* sp., *Cryptocodinium cohnii*, *Cryptomonas* sp., *Cyclotella cryptica*, *Cyclotella meneghiniana*, *Cyclotella* sp., *Dunaliella* sp., *Dunaliella bardawil*, *Dunaliella bioculata*, *Dunaliella granulate*, *Dunaliella maritime*, *Dunaliella minuta*, *Dunaliella parva*, *Dunaliella peircei*, *Dunaliella primolecta*, *Dunaliella salina*, *Dunaliella terricola*, *Dunaliella tertiolecta*, *Dunaliella viridis*, *Dunaliella tertiolecta*, *Eremosphaera viridis*, *Eremosphaera* sp., *Ellipsoidon* sp., *Euglena*, *Franceia* sp., *Fragilaria crotonensis*, *Fragilaria* sp., *Gleocapsa* sp., *Gloeothamnion* sp., *Hymenomonas* sp., *Isochrysis aff. galbana*, *Isochrysis galbana*, *Lepocinclis*, *Micractinium*, *Micractinium* (UTEX LB 2614), *Monoraphidium minutum*, *Monoraphidium* sp., *Nannochloris* sp., *Nannochloropsis salina*, *Nannochloropsis* sp., *Navicula acceptata*, *Navicula biskanterae*, *Navicula pseudotenelloides*, *Navicula pelliculosa*, *Navicula saprophila*, *Navicula* sp., *Nephrochloris* sp., *Nephroselmis* sp., *Nitzschia communis*, *Nitzschia alexandrina*, *Nitzschia communis*, *Nitzschia dissipata*, *Nitzschia frustulum*, *Nitzschia hantzschiana*, *Nitzschia inconspicua*, *Nitzschia intermedia*, *Nitzschia microcephala*, *Nitzschia pusilla*, *Nitzschia pusilla elliptica*, *Nitzschia pusilla monoensis*, *Nitzschia quadrangular*, *Nitzschia* sp., *Ochromonas* sp., *Oocystis parva*, *Oocystis pusilla*, *Oocystis* sp., *Oscillatoria limnetica*, *Oscil-*

latoria sp., *Oscillatoria subbrevis*, *ParaChlorella kessleri*, *Pascheria acidophila*, *Pavlova* sp., *Phagus*, *Phormidium*, *Platymonas* sp., *Pleurochrysis carterae*, *Pleurochrysis dentate*, *Pleurochrysis* sp., *Prototheca wickerhamii*, *Prototheca stagnora*, *Prototheca portoricensis*, *Prototheca moriformis*, *Prototheca zopfii*, *PseudoChlorella aquatica*, *Pyramimonas* sp., *Pyrobotrys*, *Rhodococcus opacus*, *Sarcinoid chryso-phyte*, *Scenedesmus armatus*, *Schizochytrium*, *Spirogyra*, *Spirulina platensis*, *Stichococcus* sp., *Synechococcus* sp., *Tetraedron*, *Tetraselmis* sp., *Tetraselmis suecica*, *Thalassiosira weissflogii*, and *Viridiella fridericiana*

Illustrative host cells feature oleaginous cells that produce altered fatty acid profiles and/or altered regiospecific distribution of fatty acids in glycerolipids, and products produced from the cells. Examples of oleaginous cells include microbial cells having a type II lipid biosynthesis pathway, including plastidic oleaginous cells such as those of oleaginous algae. Specific examples of cells include heterotrophic or obligate eukaryotic heterotrophic microalgae of the phylum Chlorophyta, the class Trebouxiophytae, the order Chlorellales, or the family Chlorellaceae. Examples of oleaginous microalgae are provided in Published PCT Patent Applications WO2008/151149, WO2010/06032, WO2011/150410, and WO2011/150411, including species of *Chlorella* and *Prototheca*, a genus comprising obligate heterotrophs. The oleaginous cells can be, for example, capable of producing 25%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, or about 90% oil by cell weight, $\pm 5\%$. The above mentioned publications also disclose methods for cultivating such cells and extracting oil, especially from microalgal cells; such methods are applicable to the cells disclosed herein. In any of the embodiments described herein, the cells can be heterotrophic cells comprising an exogenous invertase gene so as to allow the cells to produce oil from a sucrose feedstock.

Illustrative embodiments of host cells include recombinant oleaginous cells expressing one or more exogenous genes encoding fatty acid biosynthesis enzymes. As a result, some embodiments feature cell oils never before obtainable in a cell oil. In some cases, the cell oils were not obtainable from a non-plant or non-seed oil, or not obtainable at all.

The oleaginous cells produce a storage oil, which may be stored in storage vesicles of the cell. A raw cell oil may be obtained from the cells by disrupting the cells and isolating the oil. The oils produced may be refined, bleached and deodorized (RBD) as known in the art or as described in WO2010/120939. The raw or RBD oils may be used in a variety of food, chemical, and industrial products or processes. After recovery of the oil, a valuable residual biomass remains. Uses for the residual biomass include the production of paper, plastics, absorbents, adsorbents, as animal feed, for human nutrition, or for fertilizer.

Where a fatty acid profile of a triglyceride cell oil is given, it will be understood that this refers to a nonfractionated sample of the storage oil extracted from the cell analyzed under conditions in which phospholipids have been removed or with an analysis method that is substantially insensitive to the fatty acids of the phospholipids (e.g. using chromatography and mass spectrometry). Because the cells are oleaginous, in some cases the storage oil will constitute the bulk of all the TAGs in the cell.

In varying embodiments, the host cell is a plastidic cell, e.g., a heterotrophic microalgae of the phylum Chlorophyta, the class Trebouxiophytae, the order Chlorellales, or the family Chlorellaceae. In varying embodiments, the cell is oleaginous and capable of accumulating at least 40% oil by dry cell weight. The cell can be an obligate heterotroph, such

as a species of *Prototheca*, including *Prototheca moriformis* or *Prototheca zopfii*. The nucleic acid encoding the variant acyl-ACP TEs described herein can also be expressed in autotrophic algae or plants. Optionally, the cell is capable of using sucrose to produce oil and a recombinant invertase gene may be introduced to allow metabolism of sucrose, as described in PCT Publications WO2008/151149, WO2010/06032, WO2011/150410, WO2011/150411, and international patent application PCT/US12/23696. The invertase may be codon optimized and integrated into a chromosome of the cell, as may all of the genes mentioned here. Codon usage for different algal and plant species of interest is known in the art and can be found, e.g., on the internet at the Codon Usage Database at kazusa.or.jp/codon/.

The polynucleotides encoding the variant acyl-ACP TEs described herein further can be expressed in a wide variety of plant host cells. Of particular interest are plant cells of plants involved in the production of vegetable oils for edible and industrial uses, including e.g., temperate oilseed crops. Plants of interest include, but are not limited to, rapeseed (Canola and High Erucic Acid varieties), sunflower, safflower, cotton, *Cuphea*, soybean, peanut, coconut and oil palms, and corn. See, U.S. Pat. Nos. 5,850,022; 5,723,761; 5,639,790; 5,807,893; 5,455,167; 5,654,495; 5,512,482; 5,298,421; 5,667,997; and U.S. Pat. Nos. 5,344,771; 5,304,481.

Oils with Non-Naturally Occurring Fatty Acid Profiles

Oils disclosed herein are distinct from other naturally occurring oils that are high in mid-chain fatty acids, such as palm oil, palm kernel oil, and coconut oil. For example, levels of contaminants such as carotenoids are far higher in palm oil and palm kernel oil than in the oils described herein. Palm and palm kernel oils in particular contain alpha and beta carotenes and lycopene in much higher amounts than is in the oils described herein. In addition, over 20 different carotenoids are found in palm and palm kernel oil, whereas the Examples demonstrate that the oils described herein contain very few carotenoids species and very low levels. In addition, the levels of vitamin E compounds such as tocotrienols are far higher in palm, palm kernel, and coconut oil than in the oils described herein.

Generally, *Prototheca* strains have very little or no fatty acids with the chain length C8-C14. For example, *Prototheca* strains *Prototheca moriformis* (UTEX 1435), *Prototheca krugani* (UTEX 329), *Prototheca stagnora* (UTEX 1442) and *Prototheca zopfii* (UTEX 1438) produce no (or undetectable amounts) C8 fatty acids, between 0-0.01% C10 fatty acids, between 0.03-2.1% C12 fatty acids and between 1.0-1.7% C14 fatty acids.

In some cases, the oleaginous cells (e.g. *Prototheca* strains) containing a transgene encoding a variant fatty acyl-ACP thioesterase has a fatty acid profile characterized by 5-10%, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, or 90-99% C8, C10, C12, or C14 fatty acids. In other cases, the *Prototheca* strains containing a transgene encoding a fatty acyl-ACP thioesterase that has activity towards fatty acyl-ACP substrates of chain length C12 and C14 and produces fatty acids of the chain length C12 and the chain length C14 at a ratio of 1:1+/-20%.

In some instances, keeping the transgenic *Prototheca* strains under constant and high selective pressure to retain exogenous genes is advantageous due to the increase in the desired fatty acid of a specific chain length. High levels of exogenous gene retention can also be achieved by inserting exogenous genes into the nuclear chromosomes of the cells using homologous recombination vectors and methods dis-

closed herein. Recombinant cells containing exogenous genes integrated into nuclear chromosomes are also contemplated.

Microalgal oil can also include other constituents produced by the microalgae, or incorporated into the microalgal oil from the culture medium. These other constituents can be present in varying amount depending on the culture conditions used to culture the microalgae, the species of microalgae, the extraction method used to recover microalgal oil from the biomass and other factors that may affect microalgal oil composition. Non-limiting examples of such constituents include carotenoids, present from 0.1-0.4 micrograms/ml, chlorophyll present from 0-0.02 milligrams/kilogram of oil, gamma tocopherol present from 0.4-0.6 milligrams/100 grams of oil, and total tocotrienols present from 0.2-0.5 milligrams/gram of oil.

The other constituents can include, without limitation, phospholipids, tocopherols, tocotrienols, carotenoids (e.g., alpha-carotene, beta-carotene, lycopene, etc.), xanthophylls (e.g., lutein, zeaxanthin, alpha-cryptoxanthin and beta-cryptoxanthin), and various organic or inorganic compounds.

In some cases, the oil extracted from *Prototheca* species comprises no more than 0.02 mg/kg chlorophyll. In some cases, the oil extracted from *Prototheca* species comprises no more than 0.4 mcg/ml total carotenoids. In some cases the *Prototheca* oil comprises between 0.40-0.60 milligrams of gamma tocopherol per 100 grams of oil. In other cases, the *Prototheca* oil comprises between 0.2-0.5 milligrams of total tocotrienols per gram of oil.

Oils produced from host cells expressing a variant acyl-ACP thioesterase will have an isotopic profile that distinguishes it, e.g., from blended oils from other sources. The stable carbon isotope value $\delta^{13}\text{C}$ is an expression of the ratio of $^{13}\text{C}/^{12}\text{C}$ relative to a standard (e.g. PDB, carbonate of fossil skeleton of *Belemnite americana* from Peedee formation of South Carolina). The stable carbon isotope value $\delta^{13}\text{C}$ (0/00) of the oils can be related to the $\delta^{13}\text{C}$ value of the feedstock used. In some embodiments the oils are derived from oleaginous organisms heterotrophically grown on sugar derived from a C4 plant such as corn or sugarcane. In some embodiments, the $\delta^{13}\text{C}$ (0/00) of the oil is from 10 to -17 0/00 or from 13 to -16 0/00.

In varying embodiments, a host cell expressing a variant acyl-ACP thioesterase comprising all or specificity-determining residues of a specificity domain from a C10-preferring acyl-ACP thioesterase (e.g., an acyl-ACP thioesterase from *Cuphea hookeriana*), and a catalytic domain from a C12-preferring acyl-ACP thioesterase (e.g., an acyl-ACP thioesterase from *Cuphea wrightii* or *Umbellularia californica*) produces an oil comprising at least about 10% C12:0 fatty acids, and at least about 10% C14:0 fatty acids.

In varying embodiments, a host cell expressing a variant acyl-ACP thioesterase comprising all or specificity-determining residues of a modified specificity domain of a first acyl-ACP thioesterase having one or both His163→Tyr or Leu186→Pro substitutions (or at positions corresponding to His163→Tyr or Leu186→Pro of SEQ ID NO:61), and a catalytic domain of a second acyl-ACP thioesterase produces an oil comprising at least about 5%, e.g., at least about 6%, 7%, 8%, 9%, 10%, 12%, 15%, or more, C8:0 fatty acids or at least about 5%, e.g., at least about 6%, 7%, 8%, 9%, 10%, 12%, 15%, or more, C10:0 fatty acids or a C8:0/C10:0 ratio that is at least about 5%, e.g., at least about 6%, 7%, 8%, 9%, 10%, 12%, 15%, or more. As appropriate, the specificity domain can be derived from a C8:0-, C10:0- or a C12:0-preferring acyl-ACP thioesterase and independently the catalytic domain can be derived from a C8:0-, C10:0- or

a C12:0-preferring acyl-ACP thioesterase. The specificity domain and the catalytic domain can be from the same or different acyl-ACP thioesterases. In varying embodiments, a host cell expressing a variant acyl-ACP thioesterase comprising all or specificity-determining residues of a modified specificity domain from a C10-preferring acyl-ACP thioesterase (e.g., an acyl-ACP thioesterase from *Cuphea hookeriana* having one or both His163→Tyr or Leu186→Pro substitutions), and a catalytic domain from a C10-preferring acyl-ACP thioesterase (e.g., an acyl-ACP thioesterase from *Cuphea hookeriana*) produces an oil comprising at least about 5%, e.g., at least about 6%, 7%, 8%, 9%, 10%, 12%, 15%, or more, C8:0 fatty acids or at least about 5%, e.g., at least about 6%, 7%, 8%, 9%, 10%, 12%, 15%, or more, C10:0 fatty acids or a C8:0/C10:0 ratio that is at least about 5%, e.g., at least about 6%, 7%, 8%, 9%, 10%, 12%, 15%, or more.

In varying embodiments, a host cell expressing a variant acyl-ACP thioesterase comprising all or specificity-determining residues of a specificity domain from a C14-preferring acyl-ACP thioesterase (e.g., an acyl-ACP thioesterase from *Cinnamomum camphorum*), and a catalytic domain from a C12-preferring acyl-ACP thioesterase (e.g., an acyl-ACP thioesterase from *Cuphea wrightii* or *Umbellularia californica*) produces an oil comprising C12:0 fatty acids and C14:0 fatty acid at an approximate 1:1 ratio; e.g. a ratio of 1:1+/-20%.

Further, host cells expressing a variant acyl-ACP thioesterase comprising 5 or more amino acid residues extending from the C-terminus of a linker domain positioned N-terminal to the hydrophobic domain, produce an oil comprising relatively elevated mid-chain length fatty acids (e.g., C8:0, C10:0, C12:0, C14:0) in comparison to host cells expressing the same acyl-ACP thioesterase without a linker domain. In varying embodiments, host cells expressing a variant acyl-ACP thioesterase comprising 5 or more amino acid residues extending from the C-terminus of a linker domain positioned N-terminal to the hydrophobic domain, produce an oil comprising mid-chain length fatty acids increased by at least 1-fold, 2-fold, 3-fold, or more, in comparison to host cells expressing the same acyl-ACP thioesterase without a linker domain.

In a specific embodiment, a recombinant cell comprises nucleic acids operable to express a product of an exogenous gene encoding a variant acyl-ACP thioesterase exogenous gene encoding an active acyl-ACP thioesterase that catalyzes the cleavage of mid-chain fatty acids from ACP. As a result, in one embodiment, the oil produced can be characterized by a fatty acid profile elevated in C8, C10, C12, and/or C14 fatty acids and reduced in C16, C18, and C18:1 fatty acids as a result of expression of the recombinant nucleic acids. In varying embodiments, the increase in C8, C10, C12, and/or C14 fatty acids is greater than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, from 75-85%, from 70-90%, from 90-200%, from 200-300%, from 300-400%, from 400-500%, or greater than 500%.

In some embodiments, an additional genetic modification to increase the level of mid-chain fatty acids in the cell or oil of the cell includes the expression of an exogenous lysophosphatidic acid acyltransferase gene encoding an active lysophosphatidic acid acyltransferase (LPAAT) that catalyzes the transfer of a mid-chain fatty-acyl group to the sn-2 position of a substituted acylglycerol. In a specific related embodiment, both an exogenous acyl-ACP thioesterase and LPAAT are stably expressed in the cell. As a result of introducing recombinant nucleic acids into an

oleaginous cell (and especially into a plastidic microbial cell) an exogenous mid-chain-specific thioesterase and an exogenous LPAAT that catalyzes the transfer of a mid-chain fatty-acyl group to the sn-2 position of a substituted acylglycerol, the cell can be made to increase the percent of a particular mid-chain fatty acid in the triacylglycerides (TAGs) that it produces by 10, 20, 30, 40, 50, 60, 70, 80, 90-fold, or more. Introduction of the exogenous LPAAT can increase mid-chain fatty acids at the sn-2 position by 1, 2, 3, 4 fold or more compared to introducing an exogenous mid-chain preferring acyl-ACP thioesterase alone. In an embodiment, the mid-chain fatty acid is greater than 30, 40, 50, 60, 70, 80, or 90% of the TAG fatty acids produced by the cell. In various embodiments, the mid-chain fatty acid is capric, caprylic, lauric, myristic, and/or palmitic.

In varying embodiments, the gene encoding an lysophosphatidic acid acyltransferase (LPAAT) is selected from the group consisting of *Arabidopsis thaliana* 1-acyl-sn-glycerol-3-phosphate acyltransferase (GenBank Accession No. AEE85783), *Brassica juncea* 1-acyl-sn-glycerol-3-phosphate acyltransferase (GenBank Accession No. ABQ42862), *Brassica juncea* 1-acyl-sn-glycerol-3-phosphate acyltransferase (GenBank Accession No. ABM92334), *Brassica napus* 1-acyl-sn-glycerol-3-phosphate acyltransferase (GenBank Accession No. CAB09138), *Chlamydomonas reinhardtii* lysophosphatidic acid acyltransferase (GenBank Accession No. EDP02300), *Cocos nucifera* lysophosphatidic acid acyltransferase (GenBank Acc. No. AAC49119), *Limnanthes alba* lysophosphatidic acid acyltransferase (GenBank Accession No. EDP02300), *Limnanthes douglasii* 1-acyl-sn-glycerol-3-phosphate acyltransferase (putative) (GenBank Accession No. CAA88620), *Limnanthes douglasii* acyl-CoA:sn-1-acylglycerol-3-phosphate acyltransferase (GenBank Accession No. ABD62751), *Limnanthes douglasii* 1-acylglycerol-3-phosphate O-acyltransferase (GenBank Accession No. CAA58239), *Ricinus communis* 1-acyl-sn-glycerol-3-phosphate acyltransferase (GenBank Accession No. EEF39377).

Alternately, or in addition to expression of an exogenous LPAAT, the cell may comprise recombinant nucleic acids that are operable to express an exogenous KASI or KASIV enzyme and optionally to decrease or eliminate the activity of a KASII, which is particularly advantageous when a mid-chain-preferring acyl-ACP thioesterase is expressed. Engineering of *Prototheca* cells to overexpress KASI and/or KASIV enzymes in conjunction with a mid-chain preferring acyl-ACP thioesterase can generate strains in which production of C10-C12 fatty acids is at least about 40% of total fatty acids, e.g., at least about 45%, 50%, 55%, 60% or more, of total fatty acids. Mid-chain production can also be increased by suppressing the activity of KASI and/or KASII (e.g., using a knockout or knockdown). Chromosomal knockout of different alleles of *Prototheca moriformis* (UTEX 1435) KASI in conjunction with overexpression of a mid-chain preferring acyl-ACP thioesterase can achieve fatty acid profiles that are at least about 60% C10-C14 fatty acids, e.g., at least about 65%, 70%, 75%, 80%, 85% or more C10-C14 fatty acids. Elevated mid-chain fatty acids can also be achieved as a result of expression of KASI RNA hairpin polynucleotides. In addition to any of these modifications, unsaturated or polyunsaturated fatty acid production can be suppressed (e.g., by knockout or knockdown) of a SAD or FAD enzyme.

In an embodiment, one of the above described high mid-chain producing cells is further engineered to produce

a low polyunsaturated oil by knocking out or knocking down one or more fatty acyl desaturases. Accordingly, the oil produced has high stability.

The high mid-chain oils or fatty acids derived from hydrolysis of these oils may be particularly useful in food, fuel and oleochemical applications including the production of lubricants and surfactants. For example, fatty acids derived from the cells can be esterified, cracked, reduced to an aldehyde or alcohol, aminated, sulfated, sulfonated, or subjected to other chemical process known in the art.

The invention, having been described in detail above, is exemplified in the following examples, which are offered to illustrate, but not to limit, the claimed invention.

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1: Mutagenesis of *Cuphea hookeriana* FATB2

We modified the activity and specificity of a FATB2 thioesterase originally isolated from *Cuphea hookeriana* (Ch FATB2, accession U39834), using site directed mutagenesis of H163 and L186 within the enzymatic core (H163 and L186 within Ch FATB2.).

For the above examples, an expression construct was used that targeted the FATB variants and selection markers to the Thi4 (thiamine biosynthesis) locus. An antibiotic resistance gene was used to select for resistance to G418 antibiotic. The UAPA promoter was used to drive FATB. The construct is exemplified in SEQ ID NO: 9.

As disclosed in PCT/US2014/013676 we discovered that grafting the *Cuphea avigera* FATB1 (Ca FATB1) N-terminal specificity domain (FIG. 2B) onto the *Cuphea hookeriana* FATB2 (FIG. 2A) improves activity and C8-C10 ratio. *Prototheca moriformis* transformants expressing Ch FATB2 H163Y, L186P (D3130) mutants exhibited about 2 fold increase in the average C8-C10 sum as well as a shift in fatty acid profile specificity relative to the wild-type Ch FATB2 (D3042).

The His at position 163 within the Ch FATB2 (FIG. 2A) is highly conserved across FATB thioesterases. In contrast, the Leu at position 186 within the Ch FATB is rare. In other FATB's, position 186 is typically occupied by a Pro or Leu. Due to these observations and also the increased activity and shift in fatty acid profile specificity of *Prototheca moriformis* strains expressing the Ch FATB2 H163Y, L186P mutant (D3130), we identified H163 and L186 as "hot spots" for mutagenesis and performed exhaustive mutagenesis at both H163 and L186 to explore the effect of amino acid combinations on activity of the Ch FATB2 when expressed within the *Prototheca moriformis* model system. Details of the cloning system are given in PCT/US2014/013676.

Thirty-eight individual Ch FATB2 variants were generated and their effect on C8:0 and C10:0 fatty acid accumulation was quantified. Transformants with C8-C10 sum within 3 standard deviations above the wild-type Ch FATB2 control (D3598) were classified as positive and those within 3 standard deviations below were scored as negative. See Table 1. The remaining transformants were classified as neutral. As shown in Table 1, *Prototheca moriformis* transformed with six of the Ch FATB2 mutants (D3570, D3573, D3582, D3584, D3588, and D3599) accumulated C8:0-C10:0 fatty acids within 3 standard above transformants expressing the wild type Ch FATB2 (D3598) control.

TABLE 1

Analysis of FATB variants for C8-C14 fatty acid production in <i>P. moriformis</i> .*						
Ch FATB2 variant		C8:0	C10:0	C12:0	C14:0	C8-C10sum
D3565-186V	average	1.82	6.86	0.20	1.64	8.68
	STDEV	0.28	0.76	0.02	0.05	1.04
D3566-186Y	average	1.94	7.17	0.22	1.72	9.11
	STDEV	0.45	1.11	0.03	0.08	1.56
D3567-186W (negative)	average	0.78	3.98	0.16	1.66	4.76
	STDEV	0.06	0.20	0.05	0.02	0.26
D3568-186T	average	1.78	7.00	0.21	1.61	8.78
	STDEV	0.38	0.89	0.02	0.06	1.27
D3569-186S	average	1.85	7.05	0.20	1.61	8.9
	STDEV	0.32	0.75	0.02	0.06	1.07
D3570-186P (positive)	average	2.86	8.68	0.25	1.65	11.54
	STDEV	0.25	0.61	0.02	0.05	0.86
D3571-186F	average	1.46	5.98	0.19	1.62	7.44
	STDEV	0.32	0.78	0.02	0.06	1.1
D3572-186M	average	1.48	6.13	0.18	1.59	7.61
	STDEV	0.23	0.46	0.01	0.03	0.69
D3573-186K (positive)	average	2.62	8.74	0.23	1.54	11.36
	STDEV	0.66	1.33	0.03	0.05	1.99
D3574-186I	average	1.56	6.35	0.18	1.63	7.91
	STDEV	0.26	0.55	0.01	0.04	0.81
D3575-186H	average	1.97	7.38	0.19	1.60	9.35
	STDEV	0.29	0.58	0.01	0.03	0.87
D3576-186G	average	1.41	5.83	0.18	1.70	7.24
	STDEV	0.25	0.69	0.02	0.07	0.94
D3577-186E	average	2.14	7.91	0.21	1.68	10.05
	STDEV	0.54	1.29	0.03	0.08	1.83
D3578-186Q	average	2.07	7.69	0.20	1.61	9.76
	STDEV	0.46	0.96	0.02	0.06	1.42
D3579-186C	average	0.95	4.77	0.16	1.60	5.72
	STDEV	0.07	0.23	0.01	0.03	0.3
D3580-186N	average	2.25	7.93	0.21	1.55	10.18
	STDEV	0.33	0.79	0.02	0.04	1.12

TABLE 1-continued

Analysis of FATB variants for C8-C14 fatty acid production in <i>P. moriformis</i> .*						
Ch FATB2 variant		C8:0	C10:0	C12:0	C14:0	C8-C10sum
D3581-186R	average	2.21	7.74	0.22	1.63	9.95
	STDEV	0.73	1.90	0.04	0.07	2.63
D3582-186A (positive)	average	2.39	8.74	0.23	1.58	11.13
	STDEV	0.78	1.94	0.04	0.06	2.72
D3603-186D	average	1.91	7.02	0.19	1.54	8.93
	STDEV	0.41	1.14	0.02	0.05	1.55
D3583-163V (negative)	average	0.00	0.12	0.03	1.89	0.12
	STDEV	0.00	0.07	0.04	0.21	0.07
D3584-163Y (positive)	average	3.71	10.52	0.30	1.61	14.23
	STDEV	0.92	1.75	0.04	0.04	2.67
D3585-163W	average	1.11	4.88	0.18	1.67	5.99
	STDEV	0.12	0.28	0.01	0.04	0.4
D3586-163T (negative)	average	0.00	0.01	0.01	1.78	0.01
	STDEV	0.00	0.03	0.02	0.13	0.03
D3587-163P (negative)	average	0.00	0.01	0.01	1.84	0.01
	STDEV	0.00	0.03	0.03	0.14	0.03
D3588-163F (positive)	average	3.79	10.82	0.31	1.59	14.61
	STDEV	0.54	0.77	0.01	0.03	1.31
D3589-163K (negative)	average	0.00	0.01	0.06	1.79	0.01
	STDEV	0.00	0.02	0.01	0.07	0.02
D3590-163L	average	1.95	7.49	0.20	1.66	9.44
	STDEV	0.38	1.15	0.03	0.08	1.53
D3591-163I (negative)	average	0.06	0.70	0.07	1.74	0.76
	STDEV	0.02	0.15	0.01	0.11	0.17
D3592-163G (negative)	average	0.00	0.01	0.06	1.81	0.01
	STDEV	0.00	0.02	0.01	0.03	0.02
D3593-163E (negative)	average	0.00	0.02	0.06	1.99	0.02
	STDEV	0.01	0.05	0.02	0.20	0.06
D3594-163Q (negative)	average	0.06	0.69	0.07	1.74	0.75
	STDEV	0.05	0.36	0.01	0.06	0.41
D3595-163C (negative)	average	0.00	0.02	0.01	1.80	0.02
	STDEV	0.00	0.05	0.02	0.17	0.05
D3596-163R (negative)	average	0.00	0.01	0.01	1.92	0.01
	STDEV	0.00	0.04	0.02	0.36	0.04
D3597-163A (negative)	average	0.00	0.00	0.01	1.72	0
	STDEV	0.00	0.00	0.03	0.14	0
D3600-163S12 (negative)	average	0.00	0.00	0.02	1.74	0
	STDEV	0.00	0.00	0.03	0.12	0
D3601-163M (negative)	average	0.02	0.76	0.02	1.75	0.78
	STDEV	0.05	0.16	0.04	0.15	0.21
D3602-163N (negative)	average	0.00	0.00	0.01	1.74	0
	STDEV	0.00	0.00	0.02	0.07	0
D3609-163D (negative)	average	0.00	0.00	0.01	1.80	0
	STDEV	0.00	0.00	0.02	0.15	0
D3598-wild type Ch FATB2	average	1.52	6.55	0.19	1.60	8.07
	STDEV	0.19	0.62	0.02	0.11	0.81
D3599-H163Y, L186P (positive)	average	5.77	12.50	0.39	1.73	18.27
	STDEV	0.63	0.99	0.03	0.05	1.62

*12 transformants were screened per mutant. The length of lipid production under low nitrogen conditions was 3 days.

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In summary, we have shown that it is possible to increase activity and shift profile specificity within C8-C10 specific FATB thioesterases derived from *Cuphea hookeriana* by using site directed mutagenesis of H163 and L186 within the N-terminal specificity domain. We found cells expressing variants that exceeded the parent ChFATB2 sequence in terms of sum of C8:0+C10:0 production including strains that produced oils with fatty acid profiles where the C8 and C10 production exceed 9, 11, 14, of the profile.

Example 2: Identification of Double Mutants in FATB

Based on the demonstrated ability to modify the activity and specificity of a FATB2 thioesterase originally isolated from *Cuphea hookeriana* (Ch FATB2, accession U39834), using site directed mutagenesis of H163 and L186 a second round of mutagenesis was initiated. Six constructs combining the positive mutations from Rd1 (C8+C10 within 3 standard deviations above the wild-type Ch FATB2 control (D3598)) were generated (Table 2).

TABLE 2

Beneficial Mutations Constructs		
1)	163Tyr	186Lys
2)	163Tyr	186Ala
3)	163Phe	186Pro
4)	163Phe	186Lys
5)	163Phe	186Ala

For the above examples, an expression construct was used that targeted the FATB variants and selection markers to the Thi4 (thiamine biosynthesis) locus. An antibiotic resistance gene was used to select for resistance to G418 antibiotic. The UAPA promoter was used to drive FATB. The construct is exemplified in SEQ ID NO: 9.

Five individual Ch FATB2 variants were generated and their effect on C8:0 and C10:0 fatty acid accumulation was quantified. Transformants with C8-C10 sum within 3 standard deviations above the wild-type Ch FATB2 control (D3598) were classified as positive (Table 3) and those within 3 standard deviations below were scored as negative (Table 3). The remaining transformants were classified as neutral. As shown in Table 3, *Prototheca moriformis* transformed with three of the Ch FATB2 mutants (D3875, D3876, and D3885) accumulated C8:0-C10:0 fatty acids within 3 standard above transformants expressing the wild type Ch FATB2 (D3598) control.

TABLE 3

Ch FATB2 variant		C8:0	C10:0	C12:0	C14:0	C8-C10sum
D3875-163F, 186A	average	4.66	12.40	0.34	1.61	19.02
	STDEV	1.27	2.39	0.05	0.19	3.73
(positive)						
D3876-163F, 186K	average	5.25	13.12	0.36	1.55	20.28
	STDEV	1.19	2.05	0.05	0.03	3.31
(positive)						
D3877-163F, 186P	average	0.00	0.00	0.00	1.90	1.90
	STDEV	0.00	0.00	0.00	0.28	0.28
(negative)						
D3884-163Y, 186A	average	4.29	11.69	0.32	1.52	17.81
	STDEV	0.58	1.06	0.03	0.04	1.69
D3885-163Y, 186K	average	5.39	13.14	0.36	1.49	20.38
	STDEV	1.31	2.18	0.05	0.03	3.52
(positive)						

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TABLE 3-continued

Ch FATB2 variant		C8:0	C10:0	C12:0	C14:0	C8-C10sum
D3598-wild type Ch FATB2	average	1.14	5.72	0.15	1.56	8.57
	STDEV	0.27	0.77	0.06	0.05	1.12
D3599-H163Y, L186P	average	5.65	12.91	0.39	1.74	20.69
	STDEV	1.29	2.06	0.06	0.02	3.42

Example 3: Mutations at FATB Position 230

In the example below, we demonstrate the ability to modify the activity and specificity of three FATB thioesterases originally isolated from *Cuphea hookeriana* (Ch FATB2, Uniprot accession U39834), *Cuphea palustris* (Cpal FATB1, Uniprot accession Q39554, SEQ ID NO: 13) and *Cuphea avigera* FATB1 (Ca FATB1 accession R4J2L6, SEQ ID NO: 14) using site directed mutagenesis of a conserved Met within the enzymatic core (M230 within Cpal FATB1).

It has recently been reported that substitution of the conserved M230 within the Cpal FATB1 with Iso, Val, Phe or Leu will increase the enzymatic activity of this thioesterase. Because these results were obtained using *E. coli*, we performed a similar screen to see if the results could be reproduced when expressed in *Prototheca moriformis* microalgae. The wild-type and thirteen Cpal FATB1 M230 mutants were generated and their effect on C8:0 fatty acid accumulation quantified. As shown in Table 4, *Prototheca moriformis* transformed with six of the Cpal FATB1 M230 mutants (D3206, D3208, D3211, D3212, D3214, and D3215) exhibited fatty acid profiles that were similar to the non-transformed S6165 host algal strain which likely is due to the mutation inactivating the Cpal FATB1 enzyme. In contrast, *Prototheca moriformis* transformants expressing one of the remaining seven Cpal FATB1 M230 mutants accumulated C8:0 fatty acids to varying degrees above the non-transformed S6165 host. D3213 (M230P) was less effective than the wild-type Cpal FATB1 transformants (D3004), while D3207 (M230L) exhibited the same C8:0 fatty acid levels as the wild-type Cpal FATB1. D3210 (M230A), D3216 (M230T), and D3217 (M230F) all accumulated ~1-1.5% more C8:0 than the wild-type D3004. Finally, D3132 (M230I) and D3209 (M230V) exhibited a 4 fold increase in C8:0 levels compared to the D3004 wild-type. While these results share some similarity with the published data derived from expression in *E. coli*, there are some notable exceptions. For example, unlike in *E. coli*, substitution of M230 with Leu did not improve C8:0 fatty acid accumulation compared to the wild-type Cpal FATB1. In addition, replacing the M230 with an Ala or Thr increased C8:0 accumulation relative to the wild-type Cpal FATB1, which was not expected based on the *E. coli* based screen.

TABLE 4

Impact on fatty acid profiles upon expression of the wild-type Cpal FATB1 or the indicated mutant within the <i>P. moriformis</i> algal strain S6165.					
Transformant		C8:0	C10:0	C12:0	C14:0
Wild-type Cpal FATB1-D3004	average	3.67	0.52	0.21	1.43
	median	2.98	0.44	0.19	1.45
M230I-D3132	average	13.04	1.66	0.40	1.13
	median	12.13	1.53	0.37	1.15
M230K-D3206	average	0.01	0.01	0.06	1.46
	median	0.00	0.00	0.06	1.45

TABLE 4-continued

Impact on fatty acid profiles upon expression of the wild-type Cpal FATB1 or the indicated mutant within the <i>P. moriformis</i> algal strain S6165.					
Transformant		C8:0	C10:0	C12:0	C14:0
M230L-D3207	average	3.32	0.44	0.55	1.54
	median	3.45	0.44	0.56	1.53
M230G-D3208	average	0.05	0.01	0.07	1.58
	median	0.07	0.00	0.07	1.58
M230V-D3209	average	14.13	1.96	0.81	1.36
	median	14.31	1.97	0.80	1.36
M230A-D3210	average	4.06	0.35	0.47	1.82
	median	3.92	0.34	0.45	1.80
M230R-D3211	average	0.00	0.02	0.06	1.43
	median	0.00	0.00	0.06	1.44
M230H-D3212	average	0.00	0.05	0.10	1.49
	median	0.00	0.05	0.09	1.47
M230P-D3213	average	1.78	0.54	1.24	2.85
	median	1.65	0.52	1.20	2.77
M230D-D3214	average	0.00	0.03	0.05	1.50
	median	0.00	0.03	0.05	1.50
M230E-D3215	average	0.00	0.00	0.05	1.49
	median	0.00	0.00	0.05	1.46
M230T-D3216	average	5.83	0.57	0.39	1.48
	median	5.77	0.57	0.40	1.52
M230F-D3217	average	5.75	0.97	0.93	1.78
	median	5.24	0.91	0.89	1.77
S6165 parent		0	0	0	1.50

Data shown is the average and median of 12-24 individual transformants for each Cpal FATB1 expression construct.

Due to the discrepancies in outcome between the *E. coli* and *P. moriformis* expression, we explored the consequence of generating mutants at the parallel position within C8-C10 specific FATB thioesterases derived from *C. hookeriana* (Ch FATB2) and *C. avigera* (Ca FATB1). FIG. 2 shows the results of replacing the Met with Iso in the Ch FATB2 (D3455, M228I) and Lys with Met or Iso in the Ca FATB1 (D3458 and D3459, respectively). Interestingly, the transformants expressing the Ch FATB2 M228I (D3455) mutant exhibit ~50% lower total C8:0-C14:0 fatty acids compared

Ca FATB1 mutant (D3459) produced C12:0 and C14:0 fatty acids which was not observed with the wild-type or K228M Ca FATB1.

In summary, we have shown that the conclusions drawn from the *e coli* expression screen only partially agrees with our data derived from expressing the Cpal FATB1 mutants in our *P. moriformis* platform. In addition, the phenotypes observed upon substitution of the same amino acid position within the Ch FATB2 and Ca FATB1 are not what would have been expected based on the original *e coli* expression screen. Our results demonstrate that the expression platform and the thioesterase influence the outcome of a mutagenesis study.

Example 4

In addition to the results shown in Table 3 we discovered that *Prototheca moriformis* transformants expressing Ch FATB2 H163Y, L186P, and 230K (D3599) mutants exhibited a shift in fatty acid profile specificity relative to the best Ch FATB2 mutant (D3599). Therefore an additional set of mutants were generated to alter the activity and specificity of Ch FATB2, Table 5. The 228K mutation corresponds to position 230 of *Cuphea palustris* FATB1 (SEQ ID NO: 13). Residue 230 of *Cuphea palustris* FATB1 corresponds to M228 in the *Cuphea hookeriana* FATB2 and *Cuphea avigera* FATB1.

TABLE 5

Beneficial Mutations Constructs			
1)	163Tyr	186Pro	228Lys
2)	163Tyr	186Lys	228Lys
3)	163Tyr	186Ala	228Lys
4)	163Phe	186Pro	228Lys
5)	163Phe	186Lys	228Lys
6)	163Phe	186Ala	228Lys

Five individual Ch FATB2 variants were generated and their effect on C8:0 and C10:0 fatty acid accumulation was quantified.

TABLE 6

Ch FATB2 variant		C8:0	C10:0	C12:0	C14:0	C8-C10sum
D3886-163F, 186A, 228K	average	3.72	2.33	0.20	1.80	8.05
	STDEV	1.06	0.46	0.03	0.10	1.64
D3887-163F, 186K, 228K	average	5.16	2.97	0.25	1.88	10.25
	STDEV	1.34	0.61	0.04	0.12	2.11
D3888-163F, 186P, 228K	average	4.57	2.72	0.18	1.85	9.32
	STDEV	1.42	0.71	0.06	0.07	2.24
D3895-163Y, 186A, 228K	average	4.17	2.51	0.20	1.84	8.71
	STDEV	1.72	0.93	0.07	0.10	2.80
D3896-163Y, 186K, 228K	average	4.35	2.70	0.22	1.80	9.06
	STDEV	0.73	0.28	0.02	0.07	1.08
D3598-wild type Ch FATB2	average	1.14	5.72	0.15	1.56	8.57
	STDEV	0.27	0.77	0.06	0.05	1.12
D3519-H163Y, L186P, 228K	average	6.27	3.57	0.22	1.89	11.94
	STDEV	2.10	0.86	0.05	0.08	3.07

to wild-type Ch FATB2 (D3042) expression. Transformants expressing the K228M Ca FATB1 (D3458) produced ~1.5 fold greater C8:0-C14:0 fatty acid level compared to the wild-type Ca FATB1 (D3456), while the K228I Ca FATB1 (D3459) was slightly less effective than wild-type Ca FATB1 expression. Importantly, both K228M and K228I Ca FATB1 mutants exhibited a novel fatty acid preference. Both Ca FATB1 mutants accumulated a lower percent of C8:0 relative to the total C8:0-C14:0 compared to the wild-type Ca FATB1. In addition, transformants expressing the K228I

Example 5

In the example below, we demonstrate the ability to modify the activity and specificity of a FATA thioesterase originally isolated from *Garcinia mangostana* (GmFATA, accession 004792), using site directed mutagenesis targeting six amino acid positions within the enzyme. The rationale for targeting three of the amino acids (G108, S111, V193) was based on research published by Facciotti, et al., *Nat Biotechnol.* (1999) 17(6):593-7. The remaining three amino

acids (L91, G96, T156) were targeted based on research performed at Solazyme with other thioesterases.

To test the impact of each mutation on the activity of the GmFATA, the wild-type and mutant genes were cloned into a vector enabling expression within the *P. moriformis* strain 53150. Table 7 summarizes the results from a three day lipid profile screen comparing the wild-type GmFATA with the 14 mutants. Three GmFATA mutants (D3998, D4000, D4003) increased the amount of C18:0 by at least 1.5 fold compared to the wild-type protein (D3997). D3998 and D4003 were mutations that had been described by Facciotti et al (Nat-Biotech 1999) as substitutions that increased the activity of the GmFATA. In contrast, the D4000 mutation was based on research at Solazyme which demonstrated this position influenced the activity of the FATB thioesterases.

TABLE 7

Algal Strain	DNA #	SEQ ID		C14:0	C16:0	C18:0	C18:1	C18:2
		NO:	GmFATA					
<i>P. moriformis</i> S3150	—	—	—	1.63	29.82	3.08	55.95	7.22
	D3997	15	Wild-Type GmFATA	1.79	29.28	7.32	52.88	6.21
	D3998	16	S111A, V193A	1.84	28.88	11.19	49.08	6.21
	D3999	17	S111V, V193A	1.73	29.92	3.23	56.48	6.46
	D4000	18	G96A	1.76	30.19	12.66	45.99	6.01
	D4001	19	G96T	1.82	30.60	3.58	55.50	6.28
	D4002	20	G96V	1.78	29.35	3.45	56.77	6.43
	D4003	21	G108A	1.77	29.06	12.31	47.86	6.08
	D4007	25	G108V	1.81	28.78	5.71	55.05	6.26
	D4004	22	L91F	1.76	29.60	6.97	53.04	6.13
	D4005	23	L91K	1.87	28.89	4.38	56.24	6.35
	D4006	24	L91S	1.85	28.06	4.81	56.45	6.47
	D4008	26	T156F	1.81	28.71	3.65	57.35	6.31
	D4009	27	T156A	1.72	29.66	5.44	54.54	6.26
	D4010	28	T156K	1.73	29.95	3.17	56.86	6.21
	D4011	29	T156V	1.80	29.17	4.97	55.44	6.27

Example 6

Wild-type *P. moriformis* storage lipid is comprised of ~60% oleic (C18:1), ~25-30% palmitic (C16:0), and ~5-8% linoleic (C18:2) acids, with minor amounts of stearic (C18:0), myristic (C14:0), α -linolenic (C18:3a), and palmitoleic (C16:1) acids. This fatty acid profile results from the relative activities and substrate affinities of the enzymes of the endogenous fatty acid biosynthetic pathway. The introduction of *Garcinia mangostana* FATA thioesterase (GarmFATA1) gene into *P. moriformis* results in oils with increased levels of stearate (C18:0). Furthermore we demonstrated that the G96A and G108A single mutations, and the (S111A, V193A) double mutations in GarmFATA1 increased C18:0 accumulation relative to the native GarmFATA1 protein.

In the present example we assessed the thioesterase activity of a series of additional GarmFATA1 mutants. These mutants were generated by combining the above-described G96A, G108A, S111A and V193A mutations into double, triple or quadruple mutants. Specifically we tested GarmFATA1 (G96A, G108A), GarmFATA1 (G96A, S111A), GarmFATA1 (G96A, V193A), GarmFATA1 (G108A, S111A), GarmFATA1 (G108A, V193A), GarmFATA1 (G96A, G108A, S111A), GarmFATA1 (G96A, G108A, V193A), GarmFATA1 (G96A, S111A, V193A), GarmFATA1 (G108A, S111A, V193A), and GarmFATA1 (G96A, G108A, S111A, V193A) mutant combinations. GarmFATA1 (G108A) was used as a control since out of all the mutants

tested earlier this one gave the best increase in C18:0 levels over the native GARMFATA1 protein. The screen was carried out in S5780, a strain previously constructed in S5100—a high oleic base strain. S5780 was created through the targeted deletion of the dominant SAD2-1 allele, reducing the rate of conversion of C18:0 to C18:1 and overexpression of PmKASII, increasing elongation of C16:0 to C18:0. S5780 was transformed with constructs that targeted the LPAT2 gene from *T. cacao* (TcLPAT2) and the above-mentioned combinations of GarmFATA1 site-directed mutants to the FATA-1 locus. TcLPAT2 is highly specific for incorporation of unsaturated fatty acids at the sn-2 position of TAGs. The S5780 strain, containing a deletion of a stearoyl ACP desaturase (SAD) allele, was made according to the teachings in co-owned applications WO2010/063031,

WO2011/150411, and/or WO2012/106560, all of which are herein incorporated by reference.

Construct Used for the Expression of TcLPAT2 and GarmFATA1 (G96A, G108A) at PmFATA1 Locus—(pSZ5990)

In this example 55780 strain, transformed with the construct pSZ5990, was generated which express *Saccharomyces carlbergensis* SUC2 gene (allowing for their selection and growth on medium containing sucrose), a *T. cacao* LPAT2 and *G. mangostana* FATA1 (G96A, G108A) thioesterase targeted at endogenous PmFATA1 genomic region. Construct pSZ5990 introduced for expression in 55780 can be written as FATA-1 3' flank::CrTub2-ScSUC2-PmPGH: Spacer1:PmG3PDH-1-TcLPAT2-PmATP:Spacer2:Pm-SAD2-2v2-CpSAD1tp_GarmFATA1(G96A, G108A) FLAG-PmSAD2-1::FATA-1 5' flank

The sequence of the transforming DNA is provided in FIG. 1. Relevant restriction sites in the construct are indicated in lowercase, underlined bold, and are from 5'-3' BspQI, KpnI, XbaI, MfeI, BamHI, AvrII, NdeI, NsiI, AflII, EcoRI, SpeI, AscII, ClaI, SacI and BspQI respectively. BspQI sites delimit the 5' and 3' ends of the transforming DNA. Bold, lowercase sequences represent genomic DNA from *P. moriformis* that permit targeted integration at the FATA1 locus via homologous recombination. Proceeding in the 5' to 3' direction, the *Chlorella reinhardtii* Tubulin 2, driving the expression of the *S. cerevisiae* SUC2 gene is indicated by lowercase, boxed text. Uppercase italics indicate the initiator ATG and terminator TGA for SUC2, while

the coding region is indicated with lowercase italics. The *P. moriformis* Phosphoglycerate dehydratase (PGH) gene 3' UTR is indicated by lowercase underlined text followed by buffer/spacer-1 DNA sequence indicated by lowercase bold italic text. Immediately following the buffer nucleotide is an endogenous G3PDH-1 promoter of *P. moriformis*, indicated

In addition to *T. cacao* LPAT2 and *G. mangostana* FATA1 (G96A, G108A) genes targeted at PmFAFA1 locus (pSZ5990) several other constructs incorporating the various mutations described above were designed for transformation into S5780. These constructs are summarized below in Table 8:

TABLE 8

Plasmid	SEQ ID	
	NO:	Genotype
pSZ5936	47	FATA-1::CrTUB2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmSAD2-2-CpSAD1_GarmFATA1(G108A)_FLAG-PmSAD2-1::FATA-1_5'
pSZ5991	48	FATA-1_3':CrTUB2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmSAD2-2-CpSAD1_GarmFATA1(G96A, S111A)_FLAG-PmSAD2-1::FATA-1_5'
pSZ5986	49	FATA-1_3':CrTUB2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmSAD2-2-CpSAD1_GarmFATA1(G96A, V193A)_FLAG-PmSAD2-1::FATA-1_5'
pSZ5982	50	FATA-1_3':CrTUB2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmSAD2-2-CpSAD1_GarmFATA1(G108A, S111A)_FLAG-PmSAD2-1::FATA-1_5'
pSZ5983	51	FATA-1_3':CrTUB2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmSAD2-2-CpSAD1_GarmFATA1(G108A, V193A)_FLAG-PmSAD2-1::FATA-1_5'
pSZ6005	52	FATA-1_3':CrTUB2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmSAD2-2-CpSAD1_GarmFATA1(G96A, G108A, S111A)_FLAG-PmSAD2-1::FATA-1_5'
pSZ5984	53	FATA-1_3':CrTUB2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmSAD2-2-CpSAD1_GarmFATA1(G96A, G108A, V193A)_FLAG-PmSAD2-1::FATA-1_5'
pSZ6004	54	FATA-1_3':CrTUB2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmSAD2-2-CpSAD1_GarmFATA1(G96A, S111A, V193A)_FLAG-PmSAD2-1::FATA-1_5'
pSZ5985	55	FATA-1_3':CrTUB2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmSAD2-2-CpSAD1_GarmFATA1(G108A, S111A, V193A)_FLAG-PmSAD2-1::FATA-1_5'
pSZ5987	56	FATA-1_3':CrTUB2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmSAD2-2-CpSAD1_GarmFATA1(G96A, G108A, S111A, V193A)_FLAG-PmSAD2-1::FATA-1_5'
pSZ6018	47	FATA-1::CrTUB2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmACP1pCpSAD1_GarmFATA1(G108A)_FLAG-PmSAD2-1::FATA-1_5'
pSZ6019	50	FATA-1::CrTub2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmACP-P1pCpSAD1tp_GarmFATA1(G108A, S111A)_FLAG-PmSAD2-1::FATA-1
pSZ6020	51	FATA-1::CrTub2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmACP-P1pCpSAD1tp_GarmFATA1(G108A, V193A)_FLAG-PmSAD2-1::FATA-1
pSZ6021	53	FATA-1::CrTub2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmACP-P1pCpSAD1tp_GarmFATA1(G96A, G108A, V193A)_FLAG-PmSAD2-1::FATA-1
pSZ6022	55	FATA-1::CrTub2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmACP-P1pCpSAD1tp_GarmFATA1(G108, S111A, V193A)_FLAG-PmSAD2-1::FATA-1
pSZ6023	49	FATA-1::CrTub2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmACP-P1pCpSAD1tp_GarmFATA1(G96A, V193A)_FLAG-PmSAD2-1::FATA-1
pSZ6026	48	FATA-1::CrTub2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmACP-P1pCpSAD1tp_GarmFATA1(G96A, S111A)_FLAG-PmSAD2-1::FATA-1
pSZ6028	52	FATA-1::CrTub2-ScSUC2-PmPGH:PmG3PDH-1-TcLPAT2-PmATP:PmACP-P1pCpSAD1tp_GarmFATA1(G96A, G108A, S111A)_FLAG-PmSAD2-1::FATA-1

by boxed lowercase text. Uppercase italics indicate the Initiator ATG and terminator TGA codons of the *T. cacao* LPAT2 gene, while the lowercase italics indicate the remainder of the gene. The *P. moriformis* Adenosine triphosphate (ATP) gene 3' UTR is indicated by lowercase underlined text followed by the buffer/spacer 2 nucleotide sequence indicated by lowercase bold italic text. Immediately following the spacer-2 sequence is the endogenous PmSAD2-2 promoter of *P. moriformis*, indicated by boxed lowercase text. Uppercase italics indicate the initiator ATG and terminator TGA for *G. mangostana* FATA1 gene while the coding region is indicated with lowercase italics. The FATA1 gene is translationally fused to *C. protothecoides* Stearoyl ACP Desaturase-1 (CpSAD1) transit peptide at the N terminal (indicated by underlined lowercase italic text) for proper targeting to chloroplast and the 3xFLAG tag at the C terminal (indicated double underlined, italic, bold lowercase text). GarmFATA1 with CpSAD transit peptide and 3xFLAG sequence is followed by endogenous Stearoyl ACP Desaturase-1 (SAD1) gene 3' UTR indicated by lowercase underlined text. The genomic sequence of endogenous FATA1 gene is indicated by lowercase bold text. The final construct was sequenced to ensure correct reading frames and targeting sequences, and is provided as SEQ ID NO:46.

All these constructs have the same vector backbone, selectable marker, promoters, genes and 3' UTRs as pSZ5990 differing only in the mutations in the GarmFATA1. In addition, the constructs pSZ6019 to pSZ6023, pSZ6026 and pSZ6028 differ in promoter driving the particular GarmFATA1 mutant. While in pSZ5990 GarmFATA1 (G96A, G108A) is driven by PmSAD2-2v2 promoter, the various GarmFATA1 mutant combinations in pSZ6019-pSZ6028 are driven by PmACP-P1 promoter. The nucleotide sequences of various GarmFATA1 mutants used in the above constructs are shown in SEQ ID NOS: 47-56. The promoter sequence of PmACP-P1 is pSZ6019-pSZ6028 is shown in SEQ ID NO: 57. Relevant restriction sites as bold text are shown 5'-3' respectively.

To determine their impact on fatty acid profiles, all the constructs described above were transformed independently into either S5780. Primary transformants were clonally purified and grown under standard lipid production conditions at pH5.0. The resulting profiles from a set of representative clones arising from transformation of S5780 with pSZ5936 (D4933), pSZ5990 (D4950), pSZ5991 (D4951), pSZ5986 (D4948), pSZ5982 (4931), pSZ5983 (D4932), pSZ6005 (D4952), pSZ5984 (D4933), pSZ6004 (D4953), pSZ5985 (D4934), pSZ5987 (D4949), pSZ6018 (D4978),

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pSZ6019 (D4979), pSZ6020 (D4980), pSZ6021 (D4981), pSZ6022 (D4982), pSZ6023 (D4983), pSZ6026 (D4986), pSZ6028 (D4988) are shown in Tables 9-19 respectively.

Table 13 lists the average fatty acid profiles and glucose consumption (relative to the 57485 base strain) for each set of transformants. Disruption of one allele of FATA-1 reduces C16:0 by 1-2%, while TcLPAT2 activity manifests as a 1-1.5% increase in C18:2 in these strains. D4993 and D4978 expressing GarmFATA1 (G108A) mutant accumulated between 44.69% to 45.33% and 34.26 to 50.94% C18:0 respectively. D4993 has GarmFATA1 (G108A) driven by PmSAD2-2 promoter while for D4978 PmACP-P1 promoter drives the GarmFATA1 (G108A). Strains with the (G96A,

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G108A), (G108A, S111A) and (G108A, V193A) combinations consistently accumulated more C18:0 than the (G108A) single mutant, with minimal increases in C16:0. D4950 (G96A, G108A) produced more than 50% C18:0 in multiple strains. The (G96A, G108A, S111A), (G96A, G108A, V193A) and (G96A, S111A, V193A) triple mutants also produced generally higher C18:0, but at a cost of increased C16:0. The (G108A, S111A, V193A) triple mutant and (G96A, G108A, S111A, V193A) quadruple mutant produced C18:0 less than the G108 single mutant. PmACP-P1 promoter generally resulted in more C18:0 than the ones driven by PmSAD2-2 promoter.

TABLE 9

Sample ID	Fatty acid profile						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0
S5780	0.75	7.07	30.32	51.61	5.96	0.79	2.16
S5780; T1402; D4993-7	0.65	4.66	45.33	38.86	7.42	0.64	1.50
S5780; T1402; D4993-4	0.66	4.62	45.22	38.64	7.70	0.67	1.51
S5780; T1402; D4993-2	0.63	4.54	44.94	39.11	7.59	0.66	1.54
S5780; T1402; D4993-8	0.65	4.52	44.92	39.22	7.62	0.65	1.50
S5780; T1402; D4993-9	0.64	4.60	44.69	39.45	7.52	0.64	1.48
S5780; T1395; D4978-1	0.72	5.22	50.94	32.58	7.49	0.67	1.43
S5780; T1395; D4978-6	0.68	5.15	49.26	34.74	7.17	0.65	1.45
S5780; T1395; D4978-2	0.78	6.21	43.12	39.62	7.01	0.72	1.57
S5780; T1395; D4978-3	0.79	6.90	34.26	48.01	6.41	0.79	1.91

Table 9 provides primary 3-day Fatty acid profiles of representative 55780 strains transformed with D4993 (pSZ5936) and D4978 (pSZ6018). Both pSZ5936 and pSZ6018 have GarmFATA1 (G108) mutant driven by PmSAD2-2 or PmACP-P1 respectively.

TABLE 10

Sample ID	Fatty acid profile						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0
S5780	0.70	6.98	30.82	51.28	5.80	0.77	2.27
S5780; T1402; D4950-3	0.64	5.16	50.73	32.92	7.34	0.62	1.41
S5780; T1402; D4950-8	0.64	5.17	50.63	33.10	7.28	0.62	1.41
S5780; T1402; D4950-5	0.66	5.20	50.23	33.31	7.42	0.62	1.40
S5780; T1402; D4950-7	0.65	5.15	49.90	33.81	7.31	0.63	1.40
S5780; T1402; D4950-4	0.66	5.22	49.53	34.13	7.21	0.61	1.42

Table 10 provides primary 3-day Fatty acid profiles of representative 55780 strains transformed with D4950 (pSZ5990). pSZ5990 expresses GarmFATA1 (G96A, G108) mutant driven by PmSAD2-2.

TABLE 11

Sample ID	Fatty acid profile						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0
S5780	0.81	7.56	31.15	50.19	6.12	0.82	2.18
S5780; T1402; D4951-18	0.73	5.11	46.22	36.56	7.92	0.72	1.54
S5780; T1402; D4951-8	0.70	4.80	42.65	40.59	7.77	0.72	1.58
S5780; T1402; D4951-3	0.70	4.82	42.42	40.74	7.76	0.71	1.58
S5780; T1402; D4951-4	0.69	4.82	42.28	40.88	7.76	0.73	1.60
S5780; T1402; D4951-15	0.72	4.95	42.07	40.72	8.00	0.73	1.58
S5780; T1395; D4986-21	0.79	5.78	48.77	33.99	7.54	0.69	1.48
S5780; T1395; D4986-18	0.77	5.77	48.43	34.61	7.32	0.65	1.46
S5780; T1395; D4986-23	0.78	5.66	47.64	35.30	7.44	0.69	1.49
S5780; T1395; D4986-15	0.75	5.52	47.60	35.80	7.21	0.67	1.50
S5780; T1395; D4986-1	0.84	6.38	46.95	34.55	8.29	0.64	1.33

Table 11 provides Primary 3-day Fatty acid profiles of representative 55780 strains transformed with D4951 (pSZ5991) and D4986 (pSZ6026). pSZ5991 and pSZ6026 express GarmFATA1 (G96A, S111A) mutant driven by PmSAD2-2 and PmACP-P1 respectively.

TABLE 12

Sample ID	Fatty acid profile						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0
S5780	0.82	7.51	30.86	50.34	6.27	0.86	2.16
S5780; T1388; D4948-6	0.70	4.93	46.65	36.92	7.66	0.68	1.50
S5780; T1388; D4948-10	0.66	4.79	46.23	37.72	7.51	0.68	1.43
S5780; T1388; D4948-11	0.72	5.05	46.18	36.89	8.04	0.67	1.47
S5780; T1388; D4948-4	0.72	5.11	46.11	36.97	8.00	0.66	1.45
S5780; T1388; D4948-9	0.72	5.06	46.09	36.96	8.05	0.67	1.45
S5780; T1395; D4983-25	0.73	5.85	49.47	32.96	7.77	0.62	1.49
S5780; T1395; D4983-14	0.68	5.25	48.53	35.02	7.32	0.63	1.52
S5780; T1395; D4983-27	0.70	5.66	48.35	34.56	7.56	0.62	1.50
S5780; T1395; D4983-18	0.67	5.30	48.26	35.35	7.29	0.62	1.51
S5780; T1395; D4983-13	0.68	5.31	48.09	35.59	7.27	0.63	1.48

Table 12 provides primary 3-day Fatty acid profiles of representative 55780 strains transformed with D4948 (pSZ5986) and D4983 (pSZ6023). pSZ5986 and pSZ6023²⁵ express GarmFATA1 (G96A, V193A) mutant driven by PmSAD2-2 and PmACP-P1 respectively.

TABLE 13

Sample ID	Fatty acid profile						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0
S5780	0.77	7.36	30.84	50.71	6.24	0.87	2.12
S5780; T1388; D4931-25	0.70	5.13	48.48	35.40	7.25	0.64	1.43
S5780; T1388; D4931-9	0.73	5.43	48.29	34.92	7.63	0.65	1.41
S5780; T1388; D4931-13	0.72	5.24	48.13	35.22	7.64	0.66	1.45
S5780; T1388; D4931-17	0.76	5.14	48.07	35.08	7.86	0.68	1.42
S5780; T1388; D4931-12	0.73	5.33	47.91	35.27	7.65	0.67	1.42
S5780; T1395; D4979-36	0.89	6.91	50.03	31.13	7.83	0.67	1.40
S5780; T1395; D4979-5	0.77	5.88	49.65	33.24	7.25	0.68	1.48
S5780; T1395; D4979-41	0.79	6.25	49.52	33.09	7.28	0.63	1.42
S5780; T1395; D4979-39	0.82	6.36	49.43	32.49	7.66	0.66	1.48
S5780; T1395; D4979-32	0.82	6.49	49.12	32.98	7.45	0.63	1.43

Table 13 provides primary 3-day Fatty acid profiles of representative 55780 strains transformed with D4931 (pSZ5982) and D4979 (pSZ6019). pSZ5982 and pSZ6019⁴⁵ express GarmFATA1 (G108A, S111A) mutant driven by PmSAD2-2 and PmACP-P1 respectively.

Table 14 provides primary 3-day Fatty acid profiles of representative 55780 strains transformed with D4932 (pSZ5983) and D4980 (pSZ6020). pSZ5983 and pSZ6020 express GarmFATA1 (G108A, V193A) mutant driven by PmSAD2-2 and PmACP-P1 respectively.

TABLE 14

Sample ID	Fatty acid profile						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0
S5780	0.79	7.46	31.60	49.57	6.28	0.86	2.19
S5780; T1388; D4932-48	0.78	4.59	49.48	31.74	9.13	1.30	1.84
S5780; T1388; D4932-36	0.66	4.89	49.25	34.63	7.53	0.66	1.43
S5780; T1388; D4932-28	0.66	4.93	49.04	34.91	7.50	0.65	1.38
S5780; T1388; D4932-23	0.67	4.95	49.03	34.55	7.69	0.66	1.42
S5780; T1388; D4932-5	0.68	4.93	49.01	34.77	7.54	0.67	1.38
S5780; T1395; D4980-21	0.71	4.54	51.48	32.09	7.54	0.87	1.78
S5780; T1395; D4980-1	0.72	5.80	48.65	33.81	8.04	0.62	1.46
S5780; T1395; D4980-25	0.68	5.46	47.67	35.53	7.61	0.66	1.47
S5780; T1395; D4980-18	0.77	6.49	46.51	34.39	8.69	0.71	1.45
S5780; T1395; D4980-30	0.70	5.22	45.14	38.84	6.80	0.70	1.70

TABLE 15

Sample ID	Fatty acid profile						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0
S5780	0.75	7.14	30.84	51.19	5.87	0.79	2.26
S5780; T1402; D4952-9	0.77	5.68	48.96	33.44	7.70	0.68	1.50
S5780; T1402; D4952-5	0.75	5.58	48.60	33.94	7.60	0.70	1.52
S5780; T1402; D4952-1	0.75	5.62	48.59	33.94	7.63	0.69	1.51
S5780; T1402; D4952-8	0.78	5.78	48.51	33.71	7.74	0.67	1.50
S5780; T1402; D4952-10	0.77	5.65	48.35	34.15	7.59	0.70	1.52
S5780; T1395; D4988-5	0.99	8.68	48.51	31.29	7.08	0.64	1.51
S5780; T1395; D4988-7	0.75	5.50	46.63	36.68	7.41	0.69	1.43
S5780; T1395; D4988-8	0.77	5.57	46.51	36.73	7.47	0.70	1.42
S5780; T1395; D4988-3	1.12	9.63	44.06	33.16	8.33	0.76	1.57
S5780; T1395; D4988-10	1.27	11.45	43.35	31.26	8.95	0.74	1.49

Table 15 provides primary 3-day Fatty acid profiles of representative 55780 strains transformed with D4952 (pSZ6005) and D4988 (pSZ6028). pSZ6005 and pSZ6028 express GarmFATA1 (G96A, G108A, S111A) mutant driven²⁰ by PmSAD2-2 and PmACP-P1 respectively.

TABLE 16

Sample ID	Fatty acid profile						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0
S5780	0.79	7.46	31.60	49.57	6.28	0.86	2.19
S5780; T1388; D4933-12	0.67	5.48	50.40	32.85	7.31	0.63	1.36
S5780; T1388; D4933-9	0.69	5.68	50.20	32.58	7.55	0.65	1.41
S5780; T1388; D4933-8	0.66	5.46	50.07	33.20	7.35	0.63	1.39
S5780; T1388; D4933-2	0.70	5.66	49.99	32.81	7.61	0.63	1.38
S5780; T1388; D4933-5	0.85	5.84	41.97	39.70	7.94	0.97	1.36
S5780; T1395; D4981-1	0.63	5.07	37.33	46.45	6.75	0.76	2.00
S5780; T1395; D4981-3	0.71	5.70	34.96	47.88	7.02	0.86	1.88
S5780; T1395; D4981-7	0.70	5.87	34.44	48.58	6.52	0.78	2.04
S5780; T1395; D4981-4	0.75	6.18	33.78	48.83	6.61	0.83	1.98
S5780; T1395; D4981-8	0.71	6.42	33.38	49.33	6.05	0.78	2.21

Table 16 provides primary 3-day Fatty acid profiles of representative 55780 strains transformed with D4933⁴⁵ (pSZ5984) and D4981 (pSZ6021). pSZ5984 and pSZ6021 express GarmFATA1 (G96A, G108A, V193A) mutant driven by PmSAD2-2 and PmACP-P1 respectively.

TABLE 17

Sample ID	Fatty acid profile						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0
S5780	0.74	7.27	31.04	50.75	5.96	0.81	2.18
S5780; T1388; D4953-6	0.84	6.99	47.90	33.26	7.58	0.65	1.46
S5780; T1388; D4953-4	0.85	7.09	47.54	33.64	7.46	0.66	1.42
S5780; T1402; D4953-3	0.89	6.91	47.54	33.36	7.56	0.71	1.60
S5780; T1402; D4953-9	0.91	7.26	46.67	33.52	7.90	0.70	1.49
S5780; T1402; D4953-1	0.90	7.20	46.37	33.86	7.91	0.72	1.54

Table 17 provides primary 3-day Fatty acid profiles of representative 55780 strains transformed with D4953⁶⁵ (pSZ6004). pSZ6004 expresses GarmFATA1 (G96A, S111A, V193A) mutant driven by PmSAD2-2.

TABLE 18

Sample ID	Fatty acid profile						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3α	C20:0
S5780	0.78	8.24	30.24	50.34	6.00	0.79	2.23
S5780; T1402; D4934-20	0.84	7.10	46.71	34.60	7.34	0.65	1.47
S5780; T1402; D4934-15	0.78	6.76	44.01	38.09	6.88	0.65	1.59
S5780; T1402; D4934-24	1.03	10.69	39.82	33.95	11.12	0.71	1.36
S5780; T1402; D4934-14	0.77	6.83	38.68	43.31	6.51	0.71	1.88
S5780; T1402; D4934-16	0.75	6.91	35.57	46.50	6.20	0.71	1.92
S5780; T1395; D4982-1	0.00	6.19	39.51	41.35	8.23	0.78	1.92
S5780; T1395; D4982-2	0.03	7.02	35.52	46.24	6.59	0.81	1.89

Table 18 provides primary 3-day Fatty acid profiles of representative 55780 strains transformed with D4934 (pSZ5985) and D4982 (pSZ6022). pSZ5985 and pSZ6022 express GarmFATA1 (G108A, S111A, V193A) mutant driven by PmSAD2-2 and PmACP-P1 respectively.

TABLE 19

Sample ID	Fatty acid profile						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0
S5780	0.70	6.98	30.82	51.28	5.80	0.77	2.27
S5780; T1402; D4949-2	0.62	4.54	46.07	38.20	7.44	0.63	1.46
S5780; T1402; D4949-13	0.66	4.57	45.33	38.42	7.85	0.68	1.50
S5780; T1402; D4949-7	0.64	4.61	45.02	39.06	7.55	0.64	1.50
S5780; T1402; D4949-8	0.64	4.62	44.87	39.16	7.51	0.67	1.54
S5780; T1402; D4949-3	0.64	4.88	44.18	39.83	7.18	0.65	1.56

Table 19 provides primary 3-day Fatty acid profiles of representative 55780 strains transformed with D4949 (pSZ5987). pSZ5985 expresses GarmFATA1 (G96A, G108A, S111A, V193A) mutant driven by PmSAD2-2. It is understood that the examples and embodiments described herein are for illustrative purposes only and that

various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

INFORMAL SEQUENCE LISTING

Wildtype *Cuphea hookeriana* FATB2 ("ChFATB2") amino acid sequence
 SEQ ID No: 1
 MVAAAASAFFPVPAPGASPKPGKFGNWPSSLSPSFKPKSIPNGGFQVKANDSAHPKANGSAVSLKSGSL
 NTQEDTSSPPPRFLHQLPDWSRLLTAITTVFVKSKRPDMHDRKSKRPDMLVDSFGLSTVQDGLVFRQ
 SFSIRSYEIGTDRTASIEETLMNHLQETSLSLNHCKSTGILLDDGFRGLEMCKRDLIWWVVKMQIKVNRYP
 AWGDTVEINTRFSRLGKIGMGRDWLISDCNTGEILVRATSAYAMMNQKTRRLSKLPYEVHQEIVPLFVDS
 PVIEDSDLKVHKFKVKTGDSIQKGLTPGWNLDLVNQHVSNVKYIGWILESMPTFVLEVTQELCSLALEYR
 RECRGRDSVLESVTAMPSPKVGVR SQYQHLRLLEDGTAIVNGATEWRPKNAGANGAISTGKTSNGNSVS

Cuphea hookeriana C8/C10 specific FATB2 CDS
 SEQ ID No: 2
 CTGGATACCATTTCCCTGCGAAAAAATCATGGTGGCTGCTGCAGCAAGTTCGCGATTCTCCCTGTTCCA
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 CCAAGTCAATCCCAATGGCGGATTTCAAGTTAAGGCAATGACAGCGCCATCCAAAGGCTAACCGTTT
 TGCAGTTAGTCTAAAGTCTGGCAGCCTCAACACTCAGGAGGACACTTCGTCGTCCTCCCTCCGGA
 TTTCTTCCACAGTTGCTGATGAGAGTAGGCTTCTGACTGCAATCAGCACGCTGTTCGTGAAATCTAAGA
 GGCCTGACATGCATGATCGGAAATCCAAGAGCCTGACATGCTGGTGGACTCGTTTGGGTTGGAGAGTAC
 TGTTCCAGGATGGGCTCGTGTTCGACAGAGTTTTTCGATTAGGCTTATGAAATAGGCACATGATCGAAC
 GCCTCTATAGAGACACTTATGAACCACTTCGAGGAAACATCTCAATCATTGTAAGAGTACCCGGTATTC
 TCCTTGACGGCTTCGGTCGACTCTTGAAGTGTGTAAGAGGACCTCATTTGGGTGGTAATAAAAAATGCA
 GATCAAGGTGAATCGCTATCCAGCTTGGGGGATAGTGTGAGATCAATACCCGGTTCCTCCGGTTGGGG
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 GCGCGTATGCCATGATGAATCAAAGACGAGAAGACTCTCAAACTTCCATACGAGGTTCCACAGGAGAT
 AGTGCCCTTTTTGTGCGACTCTCCTGTCATTGAAGACAGTGTGTAAGAGTGCATAAGTTTAAAGTGAAG
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 ACGTGAAGTACATTGGGTGGATTCTCGAGAGTATGCCAACAGAAAGTTTGGAGACCCAGGAGCTATGCTC
 TCTCGCCCTTGAATATAGCGGGGATGCGGAAGGACAGTGTGCTGGAGTCCGTGACCGCTACCGGATCCC
 TCAAAGTTGGAGTCCGTTCTCAGTACCAGCACTTCTGCGGCTTGAGGATGGGACTGCTATCGTGAACG
 GTGCAACTGAGTGGCGCGAAGAAATGACAGGAGCTAACGGGGCGATATCAACGGGAAAGACTTCAATGG

-continued

INFORMAL SEQUENCE LISTING

AAACTCGGTCTCTTAGAAGTGTCTCGGAACCCCTCCGAGATGTGCATTTCTTTCTCCTTTTCATTTTGT
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CCTTTGTATAATAATATGGTCAGTCGTCCTTTGTATCATTTATGTTTTTACGCCATATAAT
TTTT

Amino acid sequence of Ch FATB2-D3570, pSZ4689-the algal transit peptide is underlined, the FLAG epitope tag is bolded and the P186 residue is lower-case bold

SEQ ID NO: 3

MATASTESAFNARCGDLRRSAGSGPRRPARPLPVRGRASSLSPSFKPKSIPNGGFQVKANDSAHPKA
NGSAVSLKSGSLNTQEDTSSPPRPTFLHQLPDWSRLLTAITTVFVKSKRPDMHDRKSKRPDMLVDS
FGLESTVQDGLVFRQSFIRSIEIGTDRTASIEETLMNHLQETSLNHCKSTGILLDGFGRTP**EM**CCKRD
LIWVVIKMQIKVNRYPAWGDTVEINTRFSRLGKIGMGRDWLISDCNTGEILVRATSAYAMMNQKTRR
LSKLPYEVHQEIVPLFVDSPIEDSDLKVHKFKVKTGDSIQKGLTPGWNLDVNHQVSNVKYIGWIL
ESMPTEVLETQELCSLALEYRRECGRDSVLESVTAMDPSKVGVR**SYQHLLRLEDGTAIVNGATEWR**
PKNAGANGAISIGKTSNGNSV**MDYKDHGDYKDHIDYKDDDDK***

Amino acid sequence of Ch FATB2-D3573, pSZ4692-the algal transit peptide is underlined, the FLAG epitope tag is bolded and the K186 residue is lower-case bold

SEQ ID NO: 4

MATASTESAFNARCGDLRRSAGSGPRRPARPLPVRGRASSLSPSFKPKSIPNGGFQVKANDSAHPKA
NGSAVSLKSGSLNTQEDTSSPPRPTFLHQLPDWSRLLTAITTVFVKSKRPDMHDRKSKRPDMLVDS
FGLESTVQDGLVFRQSFIRSIEIGTDRTASIEETLMNHLQETSLNHCKSTGILLDGFGR**TK**EMCKRD
LIWVVIKMQIKVNRYPAWGDTVEINTRFSRLGKIGMGRDWLISDCNTGEILVRATSAYAMMNQKTRR
LSKLPYEVHQEIVPLFVDSPIEDSDLKVHKFKVKTGDSIQKGLTPGWNLDVNHQVSNVKYIGWIL
ESMPTEVLETQELCSLALEYRRECGRDSVLESVTAMDPSKVGVR**SYQHLLRLEDGTAIVNGATEWR**
PKNAGANGAISIGKTSNGNSV**MDYKDHGDYKDHIDYKDDDDK***

Amino acid sequence of Ch FATB2-D3582, pSZ4702-the algal transit peptide is underlined, the FLAG epitope tag is bolded and the A186 residue is lower-case bold

SEQ ID NO: 5

MATASTESAFNARCGDLRRSAGSGPRRPARPLPVRGRASSLSPSFKPKSIPNGGFQVKANDSAHPKA
NGSAVSLKSGSLNTQEDTSSPPRPTFLHQLPDWSRLLTAITTVFVKSKRPDMHDRKSKRPDMLVDS
FGLESTVQDGLVFRQSFIRSIEIGTDRTASIEETLMNHLQETSLNHCKSTGILLDGFGR**TA**EMCKRD
LIWVVIKMQIKVNRYPAWGDTVEINTRFSRLGKIGMGRDWLISDCNTGEILVRATSAYAMMNQKTRR
LSKLPYEVHQEIVPLFVDSPIEDSDLKVHKFKVKTGDSIQKGLTPGWNLDVNHQVSNVKYIGWIL
ESMPTEVLETQELCSLALEYRRECGRDSVLESVTAMDPSKVGVR**SYQHLLRLEDGTAIVNGATEWR**
PKNAGANGAISIGKTSNGNSV**MDYKDHGDYKDHIDYKDDDDK***

Amino acid sequence of Ch FATB2-D3584, pSZ4704-the algal transit peptide is underlined, the FLAG epitope tag is bolded and the Y163 residue is lower-case bold

SEQ ID NO: 6

MATASTESAFNARCGDLRRSAGSGPRRPARPLPVRGRASSLSPSFKPKSIPNGGFQVKANDSAHPKA
NGSAVSLKSGSLNTQEDTSSPPRPTFLHQLPDWSRLLTAITTVFVKSKRPDMHDRKSKRPDMLVDS
FGLESTVQDGLVFRQSFIRSIEIGTDRTASIEETLMNHLQETSLNHCKSTGILLDGFGR**TL**EMCKRD
LIWVVIKMQIKVNRYPAWGDTVEINTRFSRLGKIGMGRDWLISDCNTGEILVRATSAYAMMNQKTRR
LSKLPYEVHQEIVPLFVDSPIEDSDLKVHKFKVKTGDSIQKGLTPGWNLDVNHQVSNVKYIGWIL
ESMPTEVLETQELCSLALEYRRECGRDSVLESVTAMDPSKVGVR**SYQHLLRLEDGTAIVNGATEWR**
PKNAGANGAISIGKTSNGNSV**MDYKDHGDYKDHIDYKDDDDK***

Amino acid sequence of Ch FATB2-D3588, pSZ4709-the algal transit peptide is underlined, the FLAG epitope tag is bolded and the F163 residue is lower-case bold

SEQ ID NO: 7

MATASTESAFNARCGDLRRSAGSGPRRPARPLPVRGRASSLSPSFKPKSIPNGGFQVKANDSAHPKA
NGSAVSLKSGSLNTQEDTSSPPRPTFLHQLPDWSRLLTAITTVFVKSKRPDMHDRKSKRPDMLVDS
FGLESTVQDGLVFRQSFIRSIEIGTDRTASIEETLMNHLQETSLNHCKSTGILLDGFGR**TL**EMCKRD
LIWVVIKMQIKVNRYPAWGDTVEINTRFSRLGKIGMGRDWLISDCNTGEILVRATSAYAMMNQKTRR
LSKLPYEVHQEIVPLFVDSPIEDSDLKVHKFKVKTGDSIQKGLTPGWNLDVNHQVSNVKYIGWIL
ESMPTEVLETQELCSLALEYRRECGRDSVLESVTAMDPSKVGVR**SYQHLLRLEDGTAIVNGATEWR**
PKNAGANGAISIGKTSNGNSV**MDYKDHGDYKDHIDYKDDDDK***

Amino acid sequence of the wild-type Ch FATB2-D3598, pSZ4243-the algal transit peptide is underlined, the FLAG epitope tag is bolded and the H163 and L186 residues are lower-case bold

SEQ ID NO: 8

MATASTESAFNARCGDLRRSAGSGPRRPARPLPVRGRASSLSPSFKPKSIPNGGFQVKANDSAHPKA
NGSAVSLKSGSLNTQEDTSSPPRPTFLHQLPDWSRLLTAITTVFVKSKRPDMHDRKSKRPDMLVDS
FGLESTVQDGLVFRQSFIRSIEIGTDRTASIEETLMNHLQETSLNHCKSTGILLDGFGR**TL**EMCKRD
LIWVVIKMQIKVNRYPAWGDTVEINTRFSRLGKIGMGRDWLISDCNTGEILVRATSAYAMMNQKTRR
LSKLPYEVHQEIVPLFVDSPIEDSDLKVHKFKVKTGDSIQKGLTPGWNLDVNHQVSNVKYIGWIL
ESMPTEVLETQELCSLALEYRRECGRDSVLESVTAMDPSKVGVR**SYQHLLRLEDGTAIVNGATEWR**
PKNAGANGAISIGKTSNGNSV**MDYKDHGDYKDHIDYKDDDDK***

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INFORMAL SEQUENCE LISTING

Nucleotide sequence of the ChFATB2 expression screening vector, using pSZ4243 as an example. The 5' and 3' homology arms enabling targeted integration into the Thi4 locus are noted with lowercase; the CrTUB2 promoter is noted in uppercase italic which drives expression of the Neomycin resistance gene noted with lowercase italic followed by the PmPGH 3'UTR terminator highlighted in uppercase. The PmUAPA1 promoter (noted in bold text) drives the expression of the codon optimized ChFATB2 (noted with lowercase bold text) and is terminated with the CvNR 3'UTR noted in underlined, lower case bold. Restriction cloning sites and spacer DNA fragments are noted as underlined, uppercase plain lettering.

SEQ ID NO: 9

ccctcaactgcgacgctgggaaccttctccgggagggcgatgtgctgggtttgctccttggcagc
gctctacaccgtcagatagccatgagggcggtgatggctgtgctgggtgcccacttgcctccagagacg
gcaagtgcctccatccctctgctgtgtggtggcgagcgtgcagcagtcctctgcagcagatgagcgtg
actttggccatttcacgcaactcgagtgtaacaataccatttttcttaagcaaatgactgctgattg
accagatactgtaacgctgatctcgctccagatcgcaacagatagcagccatgttgctgctgtgaa
atctggattccgaattcgaccctggcgtccatccatgcaacagatggcgacactgttacaattcc
tgtcaccatcgccatggagcaggtccacttagattcccgatcaccacgcacatctcgctaatagt
cattcgtcgtctctcgataatctcaagtgagtgatgcatggatcttggtgacagatgcccgtatgg
gtttgcccgtggctgaggggtctgcccaggcaagtaaccagctcctctccccgacaatactc
tcgagggcaagccggtcacttgcctccagattgccaataaactcaattatggcctctgtcatgcc
atccatgggtcgtgatgaatggtcacgctcgtgctcctgaccgttccccagcctctggcgtccctgcc
ccgcccaccagocccacgcccggcggcagctcgtgccaaggctgtctcggaGGTACCCTTTCTTGC
TATGACACTTCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGCGCTGATGCAACACCGA
TGATGCTTCGACCCCAAGCTCCTTCGGGGCTGCATGGGGCTCCGATGCGCTCCAGGGCGAGC
GCTGTTTAAATAGCCAGGCCCCGATTGCAAAAGCATTATAGCGAGCTACCAAGCCAATTTCAAAC
ACCTAGATCACTACCATTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCC
TCTTCCCTTTCGTTTCAGTCAACACCCGCAACTCTAGAAATATCAatgatcgagcaggacggcctcc
acgcccgttccccgcccctgggtggagcgcctgttgggtaacgactgggcccagcagaccatcgg
ctgctccgagcggccgctgttccgctgtccgcccagggcggcccgctgctgtctgctgaagaccgac
ctgtccggcggctgaacgagctgcaggacgagccgcccggctgtcctggctggccaccacggcg
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accacggcggcagcctgctgcccacaacatcatggtggagaccggcggctctccggctccatcgact
ggggcggcctgggctggcggaccgctaccaggacatcgccctggccaccggcagacatcgccgagga
gtggggggcggaggtggccgaaccgcttccctggctgtgacggcatcgccggcccgactccccagcgc
atcgccctcaccggcctgctggagcaggttctctgaCAATTGACGCCCGCGCGGCGCACTGACGTC
TTCTCTCGAGGGCGCTGTTCTGCCTTGCAGAACAGCCCTGGAGCATGCGTGATGATCGTCTCT
GGCGCCCGCGCGGGTTTGTTCGCCCTCGCGGGCGCCGCGCGCGCGGGGGCGCATTGAAATGTTG
CAAACCCACCTGACAGATTGAGGGCCAGGCAGGAAGCGCTTGAGATGGAGGTACAGGAGTCAAGT
AACTGAAAGTTTTTATGATAACTAACAAACAAGGGTCTGTTTCTGGCCAGCGAATGACAAGAACAAGA
TTCCACATTICCGTGTAGAGGCTTGCCATCGAATGTGAGCGGGCGGGCCGGGACCCGACAAAACCC
TTACGACGTGTGAGAAAACAGTGGCGGGCACTGTCCCTGTAGCCTGAAGACACAGCAGGACGATC
GGAAGCATCACAGCACAGGATCCCGCTCTCGAACAGAGCGCGCAGGAAACGCTGAAGGCTCGCC
TCTGTGCACTTCAGCGCGGATACACCAATAACACCTGACGAAATGCGCTTGGTTCTTCGTCCA
TTAGCGAAGCGTCCGGTTGACACACAGTGCACAGTGCACGTTGGCGAGGTGGCAGGTGCAATGATCGGTGGAG
CTGATGGTTCGAAACGTTACAGCCTAGGGATATCATAGCGACTGCTACCCCCGACCAATGTGCCGAG
GCAGAAATATATACAAGAGCAGATCGCAATTAGGCACATCGCTTTGCATATCCACACACTATTC
ATCGCTGCTCGGGCAAGGCTGCAAGTGTATTTTTGTGGCCAGGAGCTGAGTCCGAAAGTACAGCG
ACGACGGCGCAGGATCCGACCCCTAGACGAGCACTGTCATTTTCCAAGCACGCGCTAAATGCGCT
GAGACCGGGTCTAAATCATCCGAAAAGTGTCAAATGGCCGATTTGGGTTCCGCTAGGACAATGCGCT
GCGGATTCGCTCGAGTCCGCTGCGGCCAAAAGGCGTGGTACAGGAAGGCGCACGGGGCCAACTCT
GCGAAGCCGGGGCCGAAACGCGGACCGCCGCTTCGATCTCGGGTGTCCCCCTCGTCAATTTCTCT
CTCTCGGGTGCAGCCAGAAAGTCTGACGAGGTCACGAAATCCGGTTACGAAAAACGCGAGGCTCT
CGAAAAACGTTGAGGTTTCGCGTCTCGCCCTAGCTATTCGTATCGCCGGGTGAGACCCAGTGCAG
AAAAACCCCTGAATAACCCGGGACCGTGGTTACCGCGCCGCTGCACCAAGGGGCTTATATAAGCCC
ACACCACACTGTCTCACACCGCATTTCTCCAACCTCGCGACTTTTCGGAAGAAATGTTATCCACCT
AGTATAGACTGCCACCGACCTGACGACCTTGTGTCTTGCAGTTTGTATTGGTCCCGGCCCTCGAGCAGA
CAGATCTGGCTAGGGTTGGCTGGCCGCTCGGCACTCCCTTTAGCCGCGCGCATCCGGTTCAG
AGGTGCGATTCCGTTGTGGAGCATTTGTCATGCGCTGTGGGGTTCGTTCCGTCGCGGGCGGGTCCG
CCATGGGCGCGCACTGGCCCTAGGGTTGTTTTCGGGCCAAGCGAGCCCTCTCACCTCGTCGCC
CCCCGCAATTCCTCTCTTTCAGCCACTAGTAACAatggccaccgcaacccttctcggcgttc
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cggcaagtccaagcggcccagcatgctggtggacagcttcggcctggagtcaccogtgcaggagcggc
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aagtgaaaccgctaccggcctggggcgacaccgtggagatcaaacaccogtccagccgctgggca
agatcggcatggggcggcactggctgatctccgactgcaaccggcgagatcctgggtgcccggcacc

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cagcgccacgcatgatgaaccagaagaccgcccgcctgtccaagctgcctacgaggtgcaccag
gagatcgtgcccctgttcgtggacagccccctgacgaggactccgacctgaggtgcacaagtca
aggtgaagaccggcgacagcatccagaaggcctgacccccggctggaaacgacctggaactgaa

Amino acid sequence of the wild-type Cpal FATB1 (D3004, pSZ4241).
The algal transit peptide is underlined, the FLAG epitope tag is
bolded and the M230 residue is lower-case bold.

SEQ ID NO: 10

MATASTESAFNARCGLRRSAGSGPRRPARPLPVRGRASSLSPLKPKSI PNGGFQVKANASHPK
ANGSAVTLKSGSLNTQEDTLSSPPPRAFFNQLPDWSMLLTAITTVFVAPEKRWTFDRKSKRPNML
MDSFGLERVVQDGLVFRQSFIRS YEICADRTAS IETVMNHVQETSLNQCKSIGLLDDGFGFRSP

Amino acid sequence of the wild-type Chook FATB2 (D3042, pSZ4243).
The algal transit peptide is underlined, the FLAG epitope tag is
bolded and the M228 residue is lower-case bold.

SEQ ID NO: 11

MATASTESAFNARCGLRRSAGSGPRRPARPLPVRGRASSLSPSFKPKSI PNGGFQVKANDSAHPKA
NGSAVSLKSGSLNTQEDTSSPPPRFFLHQLPDWSRLTAITTVFVKS KRPDMHDRKSKRPDMLVDS
FGLESTVQDGLVFRQSFIRS YEIGTDRTAS IETLMNHLQETSLNHCKSTGILLDDGFGRTLEMC

Amino acid sequence of the wild-type Ca FATB1 (D3456, pSZ4532). The
algal transit peptide is underlined, the FLAG epitope tag is bolded
and the K228 residue is lower-case bold.

SEQ ID NO: 12

MATASTFSAFNARCGLRRSAGSGPRRPARPLPVRAAINSRAPKANGSAVSLKSGSLNTQEDTSS
PPPRFFLHQLPDWSRLTAITTVFVKS KRPDMHDRKSKRPDMLMDSFGLSIVQEGLEFRQSFIRS
YEIGTDRTAS IETLMNHLQETSLNHCKSTGILLDDGFGRTPEMCKRDLI WVTM KIKVNRYP

Amino acid sequence of Cuphea palustris FATB1

SEQ ID NO: 13

MVAAAASSACFPVSPGASPKPGKLGWSSLSPLKPKSI PNGGFQVKANASHPKANG
SAVILKSGSLNIQEDILSSPPPRAFFNQLPDWSMLLTAITVIVEVAPEKRWTFDRKSKR
PNMLMDSFGLERVVQDGLVFRQSFIRS YEICADRTAS IETVMNHVQETSLNQCKSIGLL
DDGFGFRSPEMCKRDLI WVTM KIKVNRYP

Amino acid sequence of Cuphea avigera FATB1

SEQ ID NO: 14

MVAAAASSAFFVSPVPGTSPKPGKFRIPWSSLSPSFKPKIPNGGLQVKANSRAHPKANG
SAVSLKSGSLNTQEDTSSPPPRFFLHQLPDWSRLTAITTVFVKS KRPDMHDRKSKRPD

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INFORMAL SEQUENCE LISTING

MLMDSFGLESIVQEGLEFRQSFIRSYSIEIGTDRITASIEITLMNYLQETSLNHCKSTGILLDD
 GFGRTPEMCKRDLIWVVTMMKIKVNRYPAWGDTVEINTWFSRLGKIGKGRDWLISDCNTG
 EILIRATSAYATMNOKTRRLSKLPYEVHQEIAPLFVDSPPVIEDNDLKLHKFEVKTGDSI
 HKGLTPGWNDLDVNOHVSINVYIGWILESMPTVELETQELCSLALAYRRECGRDSVLESV
 TAMDPKTVGGRSQYQHLRLLEDGTDIVKCRTEWRPKNPGANGAISTGKTSNGNSVS

Amino acid sequence of Gm FATA wild-type parental gene; D3997,
 pSZ5083. The algal transit peptide is underlined and the FLAG
 epitope tag is uppercase bold

SEQ ID NO: 15

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIVVSSSSSKVNPLKTEAVVSSGLA
 DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTPTMRK
 LRLIIVWTARMHIETYKYPAWSDDVVEIESWGQEGEKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
 RRLQKVDVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPQSQYKGLVPRADLDMNQHVNNV
 TYIGWVLESMPQEIIDTHELQITITLDYRRECQHDVVDSLTSPSEDAEAVFNHNGTNGSANVSAN
 DHGCRNPLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

Amino acid sequence of Gm FATA S111A, V193A mutant gene; D3998,
 pSZ5084. The algal transit peptide is underlined, the FLAG epitope
 tag is uppercase bold and the S111A, V193A residues are lower-case
 bold.

SEQ ID NO: 16

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIVVSSSSSKVNPLKTEAVVSSGLA
 DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGF**AT**PTMRK
 LRLIIVWTARMHIETYKYPAWSDDVVEIESWGQEGEKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
 RRLQKVDaDVRDEYLVHCPRELRLAFPEENNSLKKISKLEDPQSQYKGLVPRADLDMNQHVNNV
 TYIGWVLESMPQEIIDTHELQITITLDYRRECQHDVVDSLTSPSEDAEAVFNHNGTNGSANVSAN
 DHGCRNPLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

Amino acid sequence of Gm FATA S111V, V193A mutant gene; D3999,
 pSZ5085. The algal transit peptide is underlined, the FLAG epitope
 tag is uppercase bold and the S111V, V193A residues are lower-case
 bold.

SEQ ID NO: 17

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIVVSSSSSKVNPLKTEAVVSSGLA
 DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSIGGF**V**TIPTMRK
 LRLIIVWTARMHIETYKYPAWSDDVVEIESWGQEGEKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
 RRLQKVDaDVRDEYLVHCPRELRLAFPEENNSLKKISKLEDPQSQYKGLVPRADLDMNQHVNNV
 TYIGWVLESMPQEIIDTHELQITITLDYRRECQHDVVDSLTSPSEDAEAVFNHNGTNGSANVSAN
 DHGCRNPLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

Amino acid sequence of Gm FATA G96A mutant gene;
 D4000, pSZ5086. The algal transit peptide is underlined, the FLAG
 epitope tag is uppercase bold and the G96A residue is lower-case
 bold.

SEQ ID NO: 18

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIVVSSSSSKVNPLKTEAVVSSGLA
 DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVaCNHAQSVGYSTGGFSTPTMRK
 LRLIIVWTARMHIETYKYPAWSDDVVEIESWGQEGEKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
 RRLQKVDVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPQSQYKGLVPRADLDMNQHVNNV
 TYIGWVLESMPQEIIDTHELQITITLDYRRECQHDVVDSLTSPSEDAEAVFNHNGTNGSANVSAN
 DHGCRNPLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

Amino acid sequence of Gm FATA G96T mutant gene;
 D4001, pSZ5087. The algal transit peptide is underlined, the FLAG
 epitope tag is uppercase bold and the G96T residue is lower-case
 bold.

SEQ ID NO: 19

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIVVSSSSSKVNPLKTEAVVSSGLA
 DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVtCNHAQSVGYSTGGFSTPTMRK
 LRLIIVWTARMHIETYKYPAWSDDVVEIESWGQEGEKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
 RRLQKVDVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPQSQYKGLVPRADLDMNQHVNNV
 TYIGWVLESMPQEIIDTHELQITITLDYRRECQHDVVDSLTSPSEDAEAVFNHNGTNGSANVSAN
 DHGCRNPLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

Amino acid sequence of Gm FATA G96V mutant gene;
 D4002, pSZ5088. The algal transit peptide is underlined, the FLAG
 epitope tag is uppercase bold and the G96V residue is lower-case
 bold.

SEQ ID NO: 20

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIVVSSSSSKVNPLKTEAVVSSGLA
 DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVvCNHAQSVGYSIGGESTIPTMRK
 LRLIIVWTARMHIETYKYPAWSDDVVEIESWGQEGEKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
 RRLQKVDVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPQSQYKGLVPRADLDMNQHVNNV
 TYIGWVLESMPQEIIDTHELQITITLDYRRECQHDVVDSLTSPSEDAEAVFNHNGTNGSANVSAN
 DHGCRNPLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

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Amino acid sequence of Gm FATA G108A mutant gene;
D4003, pSZ5089. The algal transit peptide is underlined, the FLAG
epitope tag is uppercase bold and the G108A residue is lower-case
bold.

SEQ ID NO: 21

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLA
DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYST**AGFSTTPTMRK**
LRLIWVTARMHIETYKYPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
RRLQKVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPSQYSKLGIVPRADLDMNQHVNNV
TYIGWVLESMPQEI IDTHELQTI TLDYRRECQHDVVDVSLTSPPESEDAEAVFNHNGTNGSANV
DHGCRNFLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

Amino acid sequence of Gm FATA L91F mutant gene;
D4004, pSZ5090. The algal transit peptide is underlined, the FLAG
epitope tag is uppercase bold and the L91F residue is lower-case
bold.

SEQ ID NO: 22

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLA
DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLQEVGCNHAQSVGYSTGGFSTTPTMRK
LRLIWVTARMHIETYKYPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
RRLQKVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPSQYSKLGIVPRADLDMNQHVNNV
TYIGWVLESMPQEI IDTHELQTI TLDYRRECQHDVVDVSLTSPPESEDAEAVFNHNGTNGSANV
DHGCRNFLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

Amino acid sequence of Gm FATA L91K mutant gene;
D4005, pSZ5091. The algal transit peptide is underlined, the FLAG
epitope tag is uppercase bold and the L91K residue is lower-case
bold.

SEQ ID NO: 23

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLA
DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLQEVGCNHAQSVGYSTGGFSTTPTMRK
LRLIWVTARMHIETYKYPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
RRLQKVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPSQYSKLGIVPRADLDMNQHVNNV
TYIGWVLESMPQEI IDTHELQTI TLDYRRECQHDVVDVSLTSPPESEDAEAVFNHNGTNGSANV
DHGCRNFLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

Amino acid sequence of Gm FATA L91S mutant gene;
D4006, pSZ5092. The algal transit peptide is underlined, the FLAG
epitope tag is uppercase bold and the L91S residue is lower-case
bold.

SEQ ID NO: 24

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLA
DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLQEVGCNHAQSVGYSTGGFSTTPTMRK
LRLIWVTARMHIETYKYPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
RRLQKVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPSQYSKLGIVPRADLDMNQHVNNV
TYIGWVLESMPQEI IDTHELQTI TLDYRRECQHDVVDVSLTSPPESEDAEAVFNHNGTNGSANV
DHGCRNFLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

Amino acid sequence of Gm FATA G108V mutant gene;
D4007, pSZ5093. The algal transit peptide is underlined, the FLAG
epitope tag is uppercase bold and the G108V residue is lower-case
bold.

SEQ ID NO: 25

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLA
DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYST**AGFSTTPTMRK**
LRLIWVTARMHIETYKYPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
RRLQKVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPSQYSKLGIVPRADLDMNQHVNNV
TYIGWVLESMPQEI IDTHELQTI TLDYRRECQHDVVDVSLTSPPESEDAEAVFNHNGTNGSANV
DHGCRNFLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

Amino acid sequence of Gm FATA T156F mutant gene;
D4008, pSZ5094. The algal transit peptide is underlined, the FLAG
epitope tag is uppercase bold and the T156F residue is lower-case
bold.

SEQ ID NO: 26

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLA
DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTTPTMRK
LRLIWVTARMHIETYKYPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNQDT
RRLQKVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPSQYSKLGIVPRADLDMNQHVNNV
TYIGWVLESMPQEI IDTHELQTI TLDYRRECQHDVVDVSLTSPPESEDAEAVFNHNGTNGSANV
DHGCRNFLHLLRLRSGNGLEINRGRIEWRKKPIR**MDYKDHDGDKDHDIDYKDDDDK**

INFORMAL SEQUENCE LISTING

Amino acid sequence of Gm FATA T156A mutant gene;
D4009, pSZ5095. The algal transit peptide is underlined, the FLAG
epitope tag is uppercase bold and the T156A residue is lower-case
bold.

SEQ ID NO: 27

MATASTFSAFNARCGDLRRSAGSGPRRPARPLVVRGRAIPPRIIIVSSSSSKVNPLKTEAVVSSGLA
DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTPTMRK
LRLIIVVTARMHIEYKYPAWSDVVEIESWQGGEGKIGARRDWILRDYATGQVIGRATSKWVMNQDT
RRLQKVDVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPQSQYSKGLVPRADLDMNQHVNNV
TYIGWVLESMPQEI IDTHELQITITLDYRRECQHDVVDVSLTSPPESEDAEAVFNHNGTNGSANVAN
DHGCRNFLHLLRLRSLGNLEINRGRIEWRKKPIRMDYKDHGDKDHDIDYKDDDDK

Amino acid sequence of Gm FATA T156K mutant gene;
D4010, pSZ5096. The algal transit peptide is underlined, the FLAG
epitope tag is uppercase bold and the T156K residue is lower-case
bold.

SEQ ID NO: 28

MATASTFSAFNARCGDLRRSAGSGPRRPARPLVVRGRAIPPRIIIVSSSSSKVNPLKTEAVVSSGLA
DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTPTMRK
LRLIIVVTARMHIEYKYPAWSDVVEIESWQGGEGKIGARRDWILRDYATGQVIGRATSKWVMNQDT
RRLQKVDVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPQSQYSKGLVPRADLDMNQHVNNV
TYIGWVLESMPQEI IDTHELQITITLDYRRECQHDVVDVSLTSPPESEDAEAVFNHNGTNGSANVAN
DHGCRNFLHLLRLRSLGNLEINRGRIEWRKKPIRMDYKDHGDKDHDIDYKDDDDK

Amino acid sequence of Gm FATA T156V mutant gene;
D4011, pSZ5097. The algal transit peptide is underlined, the FLAG
epitope tag is uppercase bold and the T156V residue is lower-case
bold.

SEQ ID NO: 29

MATASTFSAFNARCGDLRRSAGSGPRRPARPLVVRGRAIPPRIIIVSSSSSKVNPLKTEAVVSSGLA
DRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTPTMRK
LRLIIVVTARMHIEYKYPAWSDVVEIESWQGGEGKIGARRDWILRDYATGQVIGRATSKWVMNQDT
RRLQKVDVDVDRDEYLVHCPRELRLAFPEENNSLKKISKLEDPQSQYSKGLVPRADLDMNQHVNNV
TYIGWVLESMPQEI IDTHELQITITLDYRRECQHDVVDVSLTSPPESEDAEAVFNHNGTNGSANVAN
DHGCRNFLHLLRLRSLGNLEINRGRIEWRKKPIRMDYKDHGDKDHDIDYKDDDDK

Nucleotide sequence of the GmFATA wild-type parental
gene expression vector (D3997, pSZ5083). The 5' and 3' homology
arms enabling targeted integration into the Thi4 locus are noted
with lowercase; the CrTUB2 promoter is noted in uppercase italic
which drives expression of the neomycin selection marker noted with
lowercase italic followed by the PmPGH 3'UTR terminator highlighted
in uppercase. The PmSAD2-1 promoter (noted in bold text) drives
the expression of the GmFATA gene (noted with lowercase bold text)
and is terminated with the CvNR 3'UTR noted in underlined, lower
case bold. Restriction cloning sites and spacer DNA fragments are
noted as underlined, uppercase plain lettering.

SEQ ID NO: 30

ccctcaactgagcagctgggaaccttctccgggagggcagatgtgcgctgggtttgacctcttggcaagc
gctctacaccgctcgagtagcagccatgaggcggatgagctgtgctcggttgccacctctccagagacg
gcaagtcgtccatctctgctgtgtggcgagcagctgcagcagctccctctgcagcagatgagcgtg
actttggccatttcacgcaactcgagtagtacaacaatccattttcttaagcaaatgactcgtgattg
accgatactgttaacgctgatttccgctccagatcgccacagatagcagccatgttgcgtctgaaa
atctggattccgaattcgacctggcctccatccatgcaacagatggcgacacttgttacaattcc
tgtaaccatcgagcagcagctccacttagattcccgatcaccacgacacatctcgtaaatagt
cattcgttcgtgtcttcgatcaatctcaagtgagtgatgcatggatcttgggtgacgatgcggtatgg
gtttggcgcctggctgaggggtctgcccaggcaagctaacccagctccctctccccgacaatactc
tcgagggcaaaagccgctcaattgcctccagattgccaataaaactcaatattggcctctgtcatgcc
atccatgggtctgatgaatggtcacgctcgtgtcctgaccgttcccagcctctggcgtccctgcc
ccgcccaccagcccacgcccggcagctcgtgccaaggctgtctoggaGGTACCCTTCTTGCGC
TATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGCATGCAACACCGA
TGATGCTTCGACCCCGAAGCTCCTTCGGGGCTGCATGGGCGCTCCGATGCGGCTCCAGGGCGAGC
GCTGTTAAATAGCCAGGCCCCGATTGCAAAGACATTATAGCGAGCTACCAAAGCCATATTCAAAC
ACCTAGACTACTACCATTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCGTAAGGGGGCGCC
TCTTCTCTTTCGTTTTCAGTCAACCCGCAAACTCTAGAATATCAatgatcgagcaggacggcctcc
acgcccgtctccccggcctctgggtggagcgcctgttcggctacgactgggcccagcagacatcgg
ctgctccgagcggcggctgtccgctgtccgcccagggcggcccctgctgtctcgtgaagaccgac
ctgtccggcgcctgaaagagctgcaggacagggcggcccctgctcctggctggccaccaccggg
tgccctggcggcggctgctggaactggtgacgagggcggcggcggcagctggctgctggcggaggt
gccccggcaggacctgctcctcccactggcccccgagagaaggtgtccatcatggccgacgcc
atggcggcctgcaaacctggaccccccaactgccccttcgaccaaaccagggccaagcaccgcatcg
agcggcccgcaccccgatggagccggcctggtggaccaggacgacctggacgaggacacaggg
ctggccccggcggagctgttcgcccctgaaggcccgatgcccagggcggcggagacctggtggtg
acccacggcagcgcctgctgcccacatcatggtggagaaacggcggcctctccggctctcatcgact
gcccggcctggcgtggcagcgcctaccaggacatcgccctggccaccggcagatcgccgagga
gctggggggagtgggcgaacccgctcctggtgctgtacggcatcgccgcccgaactcccagcgc
atcgccctctaccgctgctggacgagttctctgaCAATTGACCCCGCGCGGCACCTGACCTG

-continued

INFORMAL SEQUENCE LISTING

Nucleotide sequence of the GmFATA S111V, V193A mutant gene (D3999, pSZ5085). The promoter, 3'UTR, selection marker and targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 32

atggccaccgcatccactttctcggcggttcaatgcccgcctgcccgcacctgctcgctcggcgggct
cggggccccggcgcccagcagggccccctcccctgcccggcgcccaccatcccccccgcatcatcgt
gggtgctcctcctcctcctccaaggtgaacccccctgaagaccgagccgctgggtgctcctcggcctggcc
gaccgctgcccctgggctcctgaccgaggacggcctgtcctacaaggagaagttcatcgtgcgct
gctacgaggtgggcatcaacaagaccgcccacgctggagaccatcgccaacctgctgcaggaggtggg
ctgcaaccacgcccagtcgctgggctactccaccggcggttctcgtcaccacccccaccatgcgcaag
ctgcccctgatctgggtgaccgcccgcacatcgagatctacaagtaccccgcctggtccgacg
tggtggagatcgagtcctggggccagggcgagggcaagatcggcaccgcccgcgactggatcctgcg
cgactacgcccacggccaggtgatcggccgcccaccctccaagtgggtgatgatgaaccaggacacc
cgcccctgcagaaggtggacgcccgcagctgcccgcagcagtagtacctggtgcaactgcccgcgagctgc
gctggccttccccgagagagaacaactcctcctgaagaagatctccaagctggagaccctccca
gtactccaagctgggctgggctgcccgcgcccgcgacctggacatgaaccagcagctgaacaactg
acctacatcggctgggtgctggagtcctgccccaggagatcatcgacaccacagagctgcagacca
tcacctggactaccgcccgcagtgcccagcagcagcagtggtggactcctgacctccccgagcc
ctccgaggacgcccagggcctggttcaaccacaacggcaccacggctccgccaactgctccgccaac
gaccacggctgcccgaacttctgcaactgctgcccctgctccggcaacggcctggagatcaaccgcg
gcccacccgagtgccgcaagaagcccaccctcggactacaaggaccacgacggcgactacaagga
ccacgacatcgactacaaggacgacgacgacaagtga

Nucleotide sequence of the GmFATA G96A mutant gene (D4000, pSZ5086). The promoter, 3'UTR, selection marker and targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 33

atggccaccgcatccactttctcggcggttcaatgcccgcctgcccgcacctgctcgctcggcgggct
cggggccccggcgcccagcagggccccctcccctgcccggcgcccaccatcccccccgcatcatcgt
gggtgctcctcctcctcctccaaggtgaacccccctgaagaccgagccgctgggtgctcctcggcctggcc
gaccgctgcccctgggctcctgaccgaggacggcctgtcctacaaggagaagttcatcgtgcgct
gctacgaggtgggcatcaacaagaccgcccacgctggagaccatcgccaacctgctgcaggaggtggc
gtgcaaccacgcccagtcgctgggctactccaccggcggttctccaccacccccaccatgcgcaag
ctgcccctgatctgggtgaccgcccgcacatcgagatctacaagtaccccgcctggtccgacg
tggtggagatcgagtcctggggccagggcgagggcaagatcggcaccgcccgcgactggatcctgcg
cgactacgcccacggccaggtgatcggccgcccaccctccaagtgggtgatgatgaaccaggacacc
cgcccctgcagaaggtggacgctggagctgcccgcagcagtagtacctggtgcaactgcccgcgagctgc
gctggccttccccgagagagaacaactcctcctgaagaagatctccaagctggagaccctccca
gtactccaagctgggctgggctgcccgcgcccgcgacctggacatgaaccagcagctgaacaactg
acctacatcggctgggtgctggagtcctgccccaggagatcatcgacaccacagagctgcagacca
tcacctggactaccgcccgcagtgcccagcagcagcagtggtggactcctgacctccccgagcc
ctccgaggacgcccagggcctggttcaaccacaacggcaccacggctccgccaactgctccgccaac
gaccacggctgcccgaacttctgcaactgctgcccctgctccggcaacggcctggagatcaaccgcg
gcccacccgagtgccgcaagaagcccaccctcggactacaaggaccacgacggcgactacaagga
ccacgacatcgactacaaggacgacgacgacaagtga

Nucleotide sequence of the GmFATA G96T mutant gene (D4001, pSZ5087). The promoter, 3'UTR, selection marker and targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 34

atggccaccgcatccactttctcggcggttcaatgcccgcctgcccgcacctgctcgctcggcgggct
cggggccccggcgcccagcagggccccctcccctgcccggcgcccaccatcccccccgcatcatcgt
gggtgctcctcctcctcctccaaggtgaacccccctgaagaccgagccgctgggtgctcctcggcctggcc
gaccgctgcccctgggctcctgaccgaggacggcctgtcctacaaggagaagttcatcgtgcgct
gctacgaggtgggcatcaacaagaccgcccacgctggagaccatcgccaacctgctgcaggaggtgac
gtgcaaccacgcccagtcgctgggctactccaccggcggttctccaccacccccaccatgcgcaag
ctgcccctgatctgggtgaccgcccgcacatcgagatctacaagtaccccgcctggtccgacg
tggtggagatcgagtcctggggccagggcgagggcaagatcggcaccgcccgcgactggatcctgcg
cgactacgcccacggccaggtgatcggccgcccaccctccaagtgggtgatgatgaaccaggacacc
cgcccctgcagaaggtggacgctggagctgcccgcagcagtagtacctggtgcaactgcccgcgagctgc
gctggccttccccgagagagaacaactcctcctgaagaagatctccaagctggagaccctccca
gtactccaagctgggctgggctgcccgcgcccgcgacctggacatgaaccagcagctgaacaactg
acctacatcggctgggtgctggagtcctgccccaggagatcatcgacaccacagagctgcagacca
tcacctggactaccgcccgcagtgcccagcagcagcagtggtggactcctgacctccccgagcc
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gaccacggctgcccgaacttctgcaactgctgcccctgctccggcaacggcctggagatcaaccgcg
gcccacccgagtgccgcaagaagcccaccctcggactacaaggaccacgacggcgactacaagga
ccacgacatcgactacaaggacgacgacgacaagtga

Nucleotide sequence of the GmFATA G96V mutant gene (D4002, pSZ5088). The promoter, 3'UTR, selection marker and targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 35

atggccaccgcatccactttctcggcggttcaatgcccgcctgcccgcacctgctcgctcggcgggct
cggggccccggcgcccagcagggccccctcccctgcccggcgcccaccatcccccccgcatcatcgt
gggtgctcctcctcctcctccaaggtgaacccccctgaagaccgagccgctgggtgctcctcggcctggcc
gaccgctgcccctgggctcctgaccgaggacggcctgtcctacaaggagaagttcatcgtgcgct
gctacgaggtgggcatcaacaagaccgcccacgctggagaccatcgccaacctgctgcaggaggtgac
gtgcaaccacgcccagtcgctgggctactccaccggcggttctccaccacccccaccatgcgcaag
ctgcccctgatctgggtgaccgcccgcacatcgagatctacaagtaccccgcctggtccgacg
tggtggagatcgagtcctggggccagggcgagggcaagatcggcaccgcccgcgactggatcctgcg
cgactacgcccacggccaggtgatcggccgcccaccctccaagtgggtgatgatgaaccaggacacc
cgcccctgcagaaggtggacgctggagctgcccgcagcagtagtacctggtgcaactgcccgcgagctgc
gctggccttccccgagagagaacaactcctcctgaagaagatctccaagctggagaccctccca
gtactccaagctgggctgggctgcccgcgcccgcgacctggacatgaaccagcagctgaacaactg
acctacatcggctgggtgctggagtcctgccccaggagatcatcgacaccacagagctgcagacca
tcacctggactaccgcccgcagtgcccagcagcagcagtggtggactcctgacctccccgagcc
ctccgaggacgcccagggcctggttcaaccacaacggcaccacggctccgccaactgctccgccaac
gaccacggctgcccgaacttctgcaactgctgcccctgctccggcaacggcctggagatcaaccgcg
gcccacccgagtgccgcaagaagcccaccctcggactacaaggaccacgacggcgactacaagga
ccacgacatcgactacaaggacgacgacgacaagtga

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INFORMAL SEQUENCE LISTING

gctacgaggtgggcatcaacaagaccgcccactggagaccatcgccaacctgctgcaggaggtggt
 gtgcaaacacgcccagtcogtgggctactccaccggcggttctccaccacccccaccatgccaag
 ctgcccctgatctgggtgaccgcccgcgatgcacatcgagatctacaagtaccccgcctggccgacg
 tgggtggagatcgagtcctggggccaggcgagggcaagatcggcaccgcccgcgactggatcctgcg
 cgactacgcccacggcaggtgatcggccgcccaccctccaagtgggtgatgatgaaccaggacacc
 cgcccctgcagaaggtggacgtggacgtgcccgcgacgagtcactgggactgccccgcgagctgc
 gctggccttccccgaggagaacaactcctcctgaagaagatctccaagtggaggaccctccca
 gtactccaagctgggctgggcccgcgcccgcgactggacatgaaccagcagctgaacaactg
 acctacatcggtgggtgctggagtcctgcccaggagatcatcgacacccacgagctgcagacca
 taccctggactaccgcccgcgagtcaccagcagcagcagctgggactcctgacctccccgagcc
 ctccgaggacgcccaggccgtgtcaaccacaacggcaccacggctccgccaactgctccgccaac
 gaccagggctgcccgaactcctgcaactgctgcccctgctccggcaacggcctggagatcaaccg
 gccgaccgagtgccgcaagaagcccaccgcatggactacaaggaccacgacggcgactacaagga
 ccacgacatcgactacaaggacgacgacgacaagtga

Nucleotide sequence of the GmFATA G108A mutant gene
 (D4003, pSZ5089). The promoter, 3'UTR, selection marker and
 targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 36

atggccaccgcatccactttctcggcggtcaatgcccgcctgcccgcgacctgctgctcgctcggcggt
 cggggcccggcgcccagcgaggccctcccctgcccggcgcccaccatcccccccgcatcatcgt
 ggtgtcctcctcctcctccaaggtgaaccccctgaaaccgagggcctgggtgctcctcggcctggcc
 gaccgctgcccctgggctcctgaccgaggacggcctgctcctacaaggagaagttcatcgtgctg
 gctacgaggtgggcatcaacaagaccgcccactggagaccatcgccaacctgctgcaggaggtggg
 ctgcaaacacgcccagtcogtgggctactccaccgcccggcttctccaccacccccaccatgccaag
 ctgcccctgatctgggtgaccgcccgcgatgcacatcgagatctacaagtaccccgcctggccgacg
 tgggtggagatcgagtcctggggccaggcgagggcaagatcggcaccgcccgcgactggatcctgcg
 cgactacgcccacggccaggtgatcggccgcccaccctccaagtgggtgatgatgaaccaggacacc
 cgcccctgcagaaggtggacgtggacgtgcccgcgacgagtcactgggactgccccgcgagctgc
 gctggccttccccgaggagaacaactcctcctgaagaagatctccaagtggaggaccctccca
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 acctacatcggtgggtgctggagtcctgcccaggagatcatcgacacccacgagctgcagacca
 taccctggactaccgcccgcgagtcaccagcagcagcagctgggactcctgacctccccgagcc
 ctccgaggacgcccaggccgtgtcaaccacaacggcaccacggctccgccaactgctccgccaac
 gaccagggctgcccgaactcctgcaactgctgcccctgctccggcaacggcctggagatcaaccg
 gccgaccgagtgccgcaagaagcccaccgcatggactacaaggaccacgacggcgactacaagga
 ccacgacatcgactacaaggacgacgacgacaagtga

Nucleotide sequence of the GmFATA L91F mutant gene
 (D4004, pSZ5090). The promoter, 3'UTR, selection marker and
 targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 37

atggccaccgcatccactttctcggcggtcaatgcccgcctgcccgcgacctgctgctcgctcggcggt
 cggggcccggcgcccagcgaggccctcccctgcccggcgcccaccatcccccccgcatcatcgt
 ggtgtcctcctcctcctccaaggtgaaccccctgaaaccgagggcctgggtgctcctcggcctggcc
 gaccgctgcccctgggctcctgaccgaggacggcctgctcctacaaggagaagttcatcgtgctg
 gctacgaggtgggcatcaacaagaccgcccactggagaccatcgccaactcctgcaaggaggtggg
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 ctgcccctgatctgggtgaccgcccgcgatgcacatcgagatctacaagtaccccgcctggccgacg
 tgggtggagatcgagtcctggggccaggcgagggcaagatcggcaccgcccgcgactggatcctgcg
 cgactacgcccacggccaggtgatcggccgcccaccctccaagtgggtgatgatgaaccaggacacc
 cgcccctgcagaaggtggacgtggacgtgcccgcgacgagtcactgggactgccccgcgagctgc
 gctggccttccccgaggagaacaactcctcctgaagaagatctccaagtggaggaccctccca
 gtactccaagctgggctgggcccgcgcccgcgactggacatgaaccagcagctgaacaactg
 acctacatcggtgggtgctggagtcctgcccaggagatcatcgacacccacgagctgcagacca
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 gaccagggctgcccgaactcctgcaactgctgcccctgctccggcaacggcctggagatcaaccg
 gccgaccgagtgccgcaagaagcccaccgcatggactacaaggaccacgacggcgactacaagga
 ccacgacatcgactacaaggacgacgacgacaagtga

Nucleotide sequence of the GmFATA L91K mutant gene
 (D4005, pSZ5091). The promoter, 3'UTR, selection marker and
 targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 38

atggccaccgcatccactttctcggcggtcaatgcccgcctgcccgcgacctgctgctcgctcggcggt
 cggggcccggcgcccagcgaggccctcccctgcccggcgcccaccatcccccccgcatcatcgt
 ggtgtcctcctcctcctccaaggtgaaccccctgaaaccgagggcctgggtgctcctcggcctggcc
 gaccgctgcccctgggctcctgaccgaggacggcctgctcctacaaggagaagttcatcgtgctg
 gctacgaggtgggcatcaacaagaccgcccactggagaccatcgccaacaagctgcaggaggtggg
 ctgcaaacacgcccagtcogtgggctactccaccggcggttctccaccacccccaccatgccaag
 ctgcccctgatctgggtgaccgcccgcgatgcacatcgagatctacaagtaccccgcctggccgacg
 tgggtggagatcgagtcctggggccaggcgagggcaagatcggcaccgcccgcgactggatcctgcg
 cgactacgcccacggccaggtgatcggccgcccaccctccaagtgggtgatgatgaaccaggacacc
 cgcccctgcagaaggtggacgtggacgtgcccgcgacgagtcactgggactgccccgcgagctgc
 gctggccttccccgaggagaacaactcctcctgaagaagatctccaagtggaggaccctccca
 gtactccaagctgggctgggcccgcgcccgcgactggacatgaaccagcagctgaacaactg

- continued

INFORMAL SEQUENCE LISTING

acctacatcggtcggtgctggagtcacatgccccaggagatcatcgacacccacgagctgcagacca
tcacctggactaccgcccggagtgccagcagcagcagctggggactccctgacctccccgagcc
ctccgaggacgcccggcgggtgtcaaccacaacggcaccacggctccgccaacgtgtccgccaac
gaccacggctcccgaacttctgcaactgctgcccctgtccggcaacggcctggagatcaaccggc
gcccacccgagtgccgcaagaagcccaccgcatggactacaaggaccacgacggcgactacaagga
ccacgacatcgactacaaggacgacgacgacaagtga

Nucleotide sequence of the GmFATA L915 mutant gene (D4006, pSZ5092). The promoter, 3'UTR, selection marker and targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 39

atggccaccgcatccaactttctcggcggttcaatgcccgtcggcgacactgcgtcgctcggcgggct
ccgggccccggcggccagcggagccctcccctgcccggggcggccatcccccccgcatcatcgt
gggtgctcctcctcctccaaggtgaaccccctgaagaccgagccgctgggtgctcctcggcctggcc
gaccgctcggcctgggtcctcctgaccgagcagcggctgtcctacaaggagaagtcatcgtgctgct
gctacgaggtgggcatcaacaagaccgcccacgtggagaccatcgccaactcgtgacgaggggtggg
ctgcaaccacgcccagtcogtgggctactccaccggcggttctccaccacccccaccatgcccag
ctgcccctgatctgggtgaccgcccgcacatcgagatctacaagtaccccgcctgggtccgacg
tggtggagatcgagtcctggggccagggcgagggcaagatcggcaccgcccggcactggatcctgcg
cgactacgcccacggccaggtgatcggccggccacctccaagtggtgatgatgaaccaggacacc
cgccgctgcagaaggtggacgtggacgtgcccgcacgagtagctgggtgcaactgcccgcgagctgc
gctggccttccccgaggagaaactcctcctgaagaagatctccaagctggaggaccccccca
gtactccaagctgggctgggtgccccgcccgcgacactggacatgaaccagcagctgaacaactgtg
acctacatcggtcgggtgctggagtcacatgccccaggagatcatcgacacccacgagctgcagacca
tcacctggactaccgcccggagtgccagcagcagcagctggggactccctgacctccccgagcc
ctccgaggacgcccggcgggtgtcaaccacaacggcaccacggctccgccaacgtgtccgccaac
gaccacggctgcccgaacttctgcaactgctgcccctgtccggcaacggcctggagatcaaccggc
gcccacccgagtgccgcaagaagcccaccgcatggactacaaggaccacgacggcgactacaagga
ccacgacatcgactacaaggacgacgacgacaagtga

Nucleotide sequence of the GmFATA G108V mutant gene (D4007, pSZ5093). The promoter, 3'UTR, selection marker and targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 40

atggccaccgcatccaactttctcggcggttcaatgcccgtcggcgacactgcgtcgctcggcgggct
ccgggccccggcggccagcggagccctcccctgcccggggcggccatcccccccgcatcatcgt
gggtgctcctcctcctccaaggtgaaccccctgaagaccgagccgctgggtgctcctcggcctggcc
gaccgctcggcctgggtcctcctgaccgagcagcggctgtcctacaaggagaagtcatcgtgctgct
gctacgaggtgggcatcaacaagaccgcccacgtggagaccatcgccaactcgtgacgaggggtggg
ctgcaaccacgcccagtcogtgggctactccaccgtcgggttctccaccacccccaccatgcccag
ctgcccctgatctgggtgaccgcccgcacatcgagatctacaagtaccccgcctgggtccgacg
tggtggagatcgagtcctggggccagggcgagggcaagatcggcaccgcccggcactggatcctgcg
cgactacgcccacggccaggtgatcggccggccacctccaagtggtgatgatgaaccaggacacc
cgccgctgcagaaggtggacgtggacgtgcccgcacgagtagctgggtgcaactgcccgcgagctgc
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acctacatcggtcgggtgctggagtcacatgccccaggagatcatcgacacccacgagctgcagacca
tcacctggactaccgcccggagtgccagcagcagcagctggggactccctgacctccccgagcc
ctccgaggacgcccggcgggtgtcaaccacaacggcaccacggctccgccaacgtgtccgccaac
gaccacggctgcccgaacttctgcaactgctgcccctgtccggcaacggcctggagatcaaccggc
gcccacccgagtgccgcaagaagcccaccgcatggactacaaggaccacgacggcgactacaagga
ccacgacatcgactacaaggacgacgacgacaagtga

Nucleotide sequence of the GmFATA T156F mutant gene (D4008, pSZ5094). The promoter, 3'UTR, selection marker and targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 41

atggccaccgcatccaactttctcggcggttcaatgcccgtcggcgacactgcgtcgctcggcgggct
ccgggccccggcggccagcggagccctcccctgcccggggcggccatcccccccgcatcatcgt
gggtgctcctcctcctccaaggtgaaccccctgaagaccgagccgctgggtgctcctcggcctggcc
gaccgctcggcctgggtcctcctgaccgagcagcggctgtcctacaaggagaagtcatcgtgctgct
gctacgaggtgggcatcaacaagaccgcccacgtggagaccatcgccaactcgtgacgaggggtggg
ctgcaaccacgcccagtcogtgggctactccaccggcggttctccaccacccccaccatgcccag
ctgcccctgatctgggtgaccgcccgcacatcgagatctacaagtaccccgcctgggtccgacg
tggtggagatcgagtcctggggccagggcgagggcaagatcggctccgcccggcactggatcctgcg
cgactacgcccacggccaggtgatcggccggccacctccaagtggtgatgatgaaccaggacacc
cgccgctgcagaaggtggacgtggacgtgcccgcacgagtagctgggtgcaactgcccgcgagctgc
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ctccgaggacgcccggcgggtgtcaaccacaacggcaccacggctccgccaacgtgtccgccaac
gaccacggctgcccgaacttctgcaactgctgcccctgtccggcaacggcctggagatcaaccggc
gcccacccgagtgccgcaagaagcccaccgcatggactacaaggaccacgacggcgactacaagga
ccacgacatcgactacaaggacgacgacgacaagtga

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INFORMAL SEQUENCE LISTING

Nucleotide sequence of the GmFATA T156A mutant gene (D4009, pSZ5095). The promoter, 3'UTR, selection marker and targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 42

atggccaccgcatccactttctcggcggttcaatgcccgcctgcccgcacctgcgtcgctcggcgggct
cggggcccggcgcccagcagggcccctcccgtgcccggcgcccacccccccgcatcatcgt
ggtgtcctcctcctcctccaaggtgaaccccctgaagaccgagccgtggtgtcctcggcctggcc
gaccgctgcccctgggtcctgaccgaggacggcctgtcctacaaggagaagttcatcgtgcgt
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gaccacggctgcccgaactcctgcaactgctgcccctgctccggcaacggcctggagatcaaccgcg
gcccacccgagtgggcgcaagaagcccaccgccatggactacaaggaccacgacggcgactacaagga
ccacgacatcgactacaaggacgacgacgacaagtga

Nucleotide sequence of the GmFATA T156K mutant gene (D4010, pSZ5096). The promoter, 3'UTR, selection marker and targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 43

atggccaccgcatccactttctcggcggttcaatgcccgcctgcccgcacctgcgtcgctcggcgggct
cggggcccggcgcccagcagggcccctcccgtgcccggcgcccacccccccgcatcatcgt
ggtgtcctcctcctcctccaaggtgaaccccctgaagaccgagccgtggtgtcctcggcctggcc
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gcccacccgagtgggcgcaagaagcccaccgccatggactacaaggaccacgacggcgactacaagga
ccacgacatcgactacaaggacgacgacgacaagtga

Nucleotide sequence of the GmFATA T156V mutant gene (D4011, pSZ5097). The promoter, 3'UTR, selection marker and targeting arms are the same as described in SEQ ID NO: 30.

SEQ ID NO: 44

atggccaccgcatccactttctcggcggttcaatgcccgcctgcccgcacctgcgtcgctcggcgggct
cggggcccggcgcccagcagggcccctcccgtgcccggcgcccacccccccgcatcatcgt
ggtgtcctcctcctcctccaaggtgaaccccctgaagaccgagccgtggtgtcctcggcctggcc
gaccgctgcccctgggtcctgaccgaggacggcctgtcctacaaggagaagttcatcgtgcgt
gctacgaggtgggcatcaacaagaccgcccacgtggagaccatcgccaacctgctgcaggaggtggg
ctgcaaccacgcccagtcggtgggctactccaccggcggttctccaccacccccaccatgcgcaag
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tggtggagatcgagtcctggggccagggcgagggcaagatcggcgctgcccgcgactggatcctgcg
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cgccgctgcagaaggtggagctggagctgcccgcagcagtagtacctggtgactgccccgcgagctgc
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gaccacggctgcccgaactcctgcaactgctgcccctgctccggcaacggcctggagatcaaccgcg
gcccacccgagtgggcgcaagaagcccaccgccatggactacaaggaccacgacggcgactacaagga
ccacgacatcgactacaaggacgacgacgacaagtga

Amino acid sequence of Gm FATA wild-type parental gene; signal peptide is removed

SEQ ID NO: 45

IPPRIIVVSSSSSKVNPLKTEAVVSSGLADRLRLGSLTEDGLSYKEKFIIVRCYEVGINKTATVETIA
NLLQEVGCNHAQSVGYSIGGESTIPTMRKLRLIHWVTARMHIETYKYPAWSDVVEIESWGQEGEKIGT
RRDWILRDYATGQVIRGRATSKWVMNMQDRRLQKVDVDRDEYLVHCPRELRLAFPEENNSSLKIKS
KLEDPSQYSKGLVPRRADLDMNQHVNNVYIQLVLESMPQEIIDTHELQITLIDYRRECEQHDVVDD

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INFORMAL SEQUENCE LISTING

SLTSPPESEDAEAVFNHNGTNGSANV SANDHGCRNFLHLRLSLSGNGLEINRGRTEWRKKPTR

Nucleotide sequence of transforming DNA contained in plasmid pSZ5990 transformed into S5780

SEQ ID NO: 46

gctcttccaaactcagataatccaataaccctcctctctcctcctcatocattcagtaccccccc
ttctcttccaaagcagcaagcgttggttacagaagaacaatcggcttcgcgcaaaagtcgcccgag
cactcccgcagcggcggcggccagcggccttgccacacaggcaacgaatacatcaataggg
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aggtccccaccgagcaggaccccagcaagctcactgggtgatgtcatctccatcaacccccggcgc
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agtcgggaaacacccgctccagaagcatccggacgggggtagcgagctgtgtcgagcgcctgg

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INFORMAL SEQUENCE LISTING

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c c a t c g c c a a c c t g c t g c a g g a g g t g g c g t g c a a c c a c g c c c a g t c c g t g g g t a c t c c a c c g c c g g
c t t t c c c a c c c c c c a c c a t g c g c a a g c t g c g c t g a t c t g g g t g a c c g c c c g a t g c a c a t c g a g
a t c t a c a a g t a c c c c g c c t g g t c c g a c g t g g t g g a g a t c g a g t c c t g g g g c c a g g g c g a g g g c a a g a
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cgtttagtggagcagcgcactccattcagctaccagtcgaactcagtgccacagtgactcccgctctt
c

Nucleotide sequence of CpSAD transit peptide fused to GarmFATA1 (G108A) gene and 3X FLAG tag in pSZ5936 and pSZ6018
SEQ ID NO: 47

actagtATGgccaccgcatccactttctcggcgttcaatgccccgtcgcggcgaacctgcgtcgctcgg
cgggctccgggcccggcggcccagcagggcccctcccctgcccggggcgcgccatcccccccgcac
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Nucleotide sequence of CpSAD transit peptide fused to GarmFATA1 (G96A, S111A) gene and 3X FLAG tag in pSZ5991 and pSZ6026
SEQ ID NO: 48

actagtATGgccaccgcatccactttctcggcgttcaatgccccgtcgcggcgaacctgcgtcgctcgg
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INFORMAL SEQUENCE LISTING

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caagqaccacgacatcgactacaagqacgacgacgacaagTGAatcgat

Nucleotide sequence of CpSAD transit peptide fused
to GarmFATA1 (G96A, V193A) gene and 3X FLAG tag in pSZ5986 and
pSZ6023

SEQ ID NO: 49

actagtATGgcccaccgcatccactttctcggcgttcaatgcccqctgcccgcacctcgctcgctcgg
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caacrqaccacgacatcgactacaagqacgacgacgacaagTGAatcgat

Nucleotide sequence of CpSAD transit peptide fused
to GarmFATA1 (G108A, S111A) gene and 3X FLAG tag in pSZ5982 and
pSZ6019

SEQ ID NO: 50

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caagqaccacgacatcgactacaagqacgacgacgacaagTGAatcgat

Nucleotide sequence of CpSAD transit peptide fused
to GarmFATA1 (G108A, V193A) gene and 3X FLAG tag in pSZ5983 and
pSZ6020

SEQ ID NO: 51

actagtATGgcccaccgcatccactttctcggcgttcaatgcccqctgcccgcacctcgctcgctcgg
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INFORMAL SEQUENCE LISTING

Nucleotide sequence of CpSAD transit peptide fused to GarmFATA1 (G96A, G108A, S111A) gene and 3X FLAG tag in pSZ6005 and pSZ6028

SEQ ID NO: 52

actagtATGgccaccgcatccactttctcggcgttcaatgcccgctgcggcgacctgcgtcgctcgg
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caaggaccacgacatcgactacaaggacgacgacgacaagTGAatcgat

Nucleotide sequence of CpSAD transit peptide fused to GarmFATA1 (G96A, G108A, V193A) gene and 3X FLAG tag in pSZ5984 and pSZ6021

SEQ ID NO: 53

actagtATGgccaccgcatccactttctcggcgttcaatgcccgctgcggcgacctgcgtcgctcgg
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caaggaccacgacatcgactacaaggacgacgacgacaagTGAatcgat

Nucleotide sequence of CpSAD transit peptide fused to GarmFATA1 (G96A, S111A, V193A) gene and 3X FLAG tag in pSZ6004

SEQ ID NO: 54

actagtATGgccaccgcatccactttctcggcgttcaatgcccgctgcggcgacctgcgtcgctcgg
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caacrgaccacgacatcgactacaaggacgacgacgacaagTGAatcgat

Nucleotide sequence of CpSAD transit peptide fused to GarmFATA1 (G108A, S111A, V193A) gene and 3X FLAG tag in pSZ5985 and pSZ6022

SEQ ID NO: 55

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Nucleotide sequence of CpSAD transit peptide fused
to GarmFATA1 (G96A, G108A, S111A, V193A) gene and 3X FLAG tag in
pSZ5987

SEQ ID NO: 56

actagtATGgcccacgcatccactttctcggcgttcaatgcccgtgcccggacactcggctcgg
cgggctccggggcccggcggccagcagggccctcccgtgcccggggcgccatcccccccgcat
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Nucleotide sequence of PmACP-P1 promoter in pSZ6019-pSZ6023, pSZ6026
and pSZ6028

SEQ ID NO: 57

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SEQUENCE LISTING

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<210> SEQ ID NO 1
<211> LENGTH: 415
<212> TYPE: PRT
<213> ORGANISM: Cuphea hookeriana

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<400> SEQUENCE: 1

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 35 40 45
 Lys Ala Asn Asp Ser Ala His Pro Lys Ala Asn Gly Ser Ala Val Ser
 50 55 60
 Leu Lys Ser Gly Ser Leu Asn Thr Gln Glu Asp Thr Ser Ser Ser Pro
 65 70 75 80
 Pro Pro Arg Thr Phe Leu His Gln Leu Pro Asp Trp Ser Arg Leu Leu
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 Thr Ala Ile Thr Thr Val Phe Val Lys Ser Lys Arg Pro Asp Met His
 100 105 110
 Asp Arg Lys Ser Lys Arg Pro Asp Met Leu Val Asp Ser Phe Gly Leu
 115 120 125
 Glu Ser Thr Val Gln Asp Gly Leu Val Phe Arg Gln Ser Phe Ser Ile
 130 135 140
 Arg Ser Tyr Glu Ile Gly Thr Asp Arg Thr Ala Ser Ile Glu Thr Leu
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 Met Asn His Leu Gln Glu Thr Ser Leu Asn His Cys Lys Ser Thr Gly
 165 170 175
 Ile Leu Leu Asp Gly Phe Gly Arg Thr Leu Glu Met Cys Lys Arg Asp
 180 185 190
 Leu Ile Trp Val Val Ile Lys Met Gln Ile Lys Val Asn Arg Tyr Pro
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 Ala Trp Gly Asp Thr Val Glu Ile Asn Thr Arg Phe Ser Arg Leu Gly
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 Lys Ile Gly Met Gly Arg Asp Trp Leu Ile Ser Asp Cys Asn Thr Gly
 225 230 235 240
 Glu Ile Leu Val Arg Ala Thr Ser Ala Tyr Ala Met Met Asn Gln Lys
 245 250 255
 Thr Arg Arg Leu Ser Lys Leu Pro Tyr Glu Val His Gln Glu Ile Val
 260 265 270
 Pro Leu Phe Val Asp Ser Pro Val Ile Glu Asp Ser Asp Leu Lys Val
 275 280 285
 His Lys Phe Lys Val Lys Thr Gly Asp Ser Ile Gln Lys Gly Leu Thr
 290 295 300
 Pro Gly Trp Asn Asp Leu Asp Val Asn Gln His Val Ser Asn Val Lys
 305 310 315 320
 Tyr Ile Gly Trp Ile Leu Glu Ser Met Pro Thr Glu Val Leu Glu Thr
 325 330 335
 Gln Glu Leu Cys Ser Leu Ala Leu Glu Tyr Arg Arg Glu Cys Gly Arg
 340 345 350
 Asp Ser Val Leu Glu Ser Val Thr Ala Met Asp Pro Ser Lys Val Gly
 355 360 365
 Val Arg Ser Gln Tyr Gln His Leu Leu Arg Leu Glu Asp Gly Thr Ala
 370 375 380
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<211> LENGTH: 1474			
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<210> SEQ ID NO 3
 <211> LENGTH: 447
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 3
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 35 40 45

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Ser Ile Pro Asn Gly Gly Phe Gln Val Lys Ala Asn Asp Ser Ala His
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 Pro Lys Ala Asn Gly Ser Ala Val Ser Leu Lys Ser Gly Ser Leu Asn
 65 70 75 80
 Thr Gln Glu Asp Thr Ser Ser Ser Pro Pro Pro Arg Thr Phe Leu His
 85 90 95
 Gln Leu Pro Asp Trp Ser Arg Leu Leu Thr Ala Ile Thr Thr Val Phe
 100 105 110
 Val Lys Ser Lys Arg Pro Asp Met His Asp Arg Lys Ser Lys Arg Pro
 115 120 125
 Asp Met Leu Val Asp Ser Phe Gly Leu Glu Ser Thr Val Gln Asp Gly
 130 135 140
 Leu Val Phe Arg Gln Ser Phe Ser Ile Arg Ser Tyr Glu Ile Gly Thr
 145 150 155 160
 Asp Arg Thr Ala Ser Ile Glu Thr Leu Met Asn His Leu Gln Glu Thr
 165 170 175
 Ser Leu Asn His Cys Lys Ser Thr Gly Ile Leu Leu Asp Gly Phe Gly
 180 185 190
 Arg Thr Pro Glu Met Cys Lys Arg Asp Leu Ile Trp Val Val Ile Lys
 195 200 205
 Met Gln Ile Lys Val Asn Arg Tyr Pro Ala Trp Gly Asp Thr Val Glu
 210 215 220
 Ile Asn Thr Arg Phe Ser Arg Leu Gly Lys Ile Gly Met Gly Arg Asp
 225 230 235 240
 Trp Leu Ile Ser Asp Cys Asn Thr Gly Glu Ile Leu Val Arg Ala Thr
 245 250 255
 Ser Ala Tyr Ala Met Met Asn Gln Lys Thr Arg Arg Leu Ser Lys Leu
 260 265 270
 Pro Tyr Glu Val His Gln Glu Ile Val Pro Leu Phe Val Asp Ser Pro
 275 280 285
 Val Ile Glu Asp Ser Asp Leu Lys Val His Lys Phe Lys Val Lys Thr
 290 295 300
 Gly Asp Ser Ile Gln Lys Gly Leu Thr Pro Gly Trp Asn Asp Leu Asp
 305 310 315 320
 Val Asn Gln His Val Ser Asn Val Lys Tyr Ile Gly Trp Ile Leu Glu
 325 330 335
 Ser Met Pro Thr Glu Val Leu Glu Thr Gln Glu Leu Cys Ser Leu Ala
 340 345 350
 Leu Glu Tyr Arg Arg Glu Cys Gly Arg Asp Ser Val Leu Glu Ser Val
 355 360 365
 Thr Ala Met Asp Pro Ser Lys Val Gly Val Arg Ser Gln Tyr Gln His
 370 375 380
 Leu Leu Arg Leu Glu Asp Gly Thr Ala Ile Val Asn Gly Ala Thr Glu
 385 390 395 400
 Trp Arg Pro Lys Asn Ala Gly Ala Asn Gly Ala Ile Ser Thr Gly Lys
 405 410 415
 Thr Ser Asn Gly Asn Ser Val Ser Met Asp Tyr Lys Asp His Asp Gly
 420 425 430
 Asp Tyr Lys Asp His Asp Ile Asp Tyr Lys Asp Asp Asp Asp Lys
 435 440 445

<210> SEQ ID NO 4

<211> LENGTH: 447

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 4

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
1          5          10          15
Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
20          25          30
Pro Val Arg Gly Arg Ala Ser Ser Leu Ser Pro Ser Phe Lys Pro Lys
35          40          45
Ser Ile Pro Asn Gly Gly Phe Gln Val Lys Ala Asn Asp Ser Ala His
50          55          60
Pro Lys Ala Asn Gly Ser Ala Val Ser Leu Lys Ser Gly Ser Leu Asn
65          70          75          80
Thr Gln Glu Asp Thr Ser Ser Ser Pro Pro Pro Arg Thr Phe Leu His
85          90          95
Gln Leu Pro Asp Trp Ser Arg Leu Leu Thr Ala Ile Thr Thr Val Phe
100         105         110
Val Lys Ser Lys Arg Pro Asp Met His Asp Arg Lys Ser Lys Arg Pro
115         120         125
Asp Met Leu Val Asp Ser Phe Gly Leu Glu Ser Thr Val Gln Asp Gly
130         135         140
Leu Val Phe Arg Gln Ser Phe Ser Ile Arg Ser Tyr Glu Ile Gly Thr
145         150         155         160
Asp Arg Thr Ala Ser Ile Glu Thr Leu Met Asn His Leu Gln Glu Thr
165         170         175
Ser Leu Asn His Cys Lys Ser Thr Gly Ile Leu Leu Asp Gly Phe Gly
180         185         190
Arg Thr Lys Glu Met Cys Lys Arg Asp Leu Ile Trp Val Val Ile Lys
195         200         205
Met Gln Ile Lys Val Asn Arg Tyr Pro Ala Trp Gly Asp Thr Val Glu
210         215         220
Ile Asn Thr Arg Phe Ser Arg Leu Gly Lys Ile Gly Met Gly Arg Asp
225         230         235         240
Trp Leu Ile Ser Asp Cys Asn Thr Gly Glu Ile Leu Val Arg Ala Thr
245         250         255
Ser Ala Tyr Ala Met Met Asn Gln Lys Thr Arg Arg Leu Ser Lys Leu
260         265         270
Pro Tyr Glu Val His Gln Glu Ile Val Pro Leu Phe Val Asp Ser Pro
275         280         285
Val Ile Glu Asp Ser Asp Leu Lys Val His Lys Phe Lys Val Lys Thr
290         295         300
Gly Asp Ser Ile Gln Lys Gly Leu Thr Pro Gly Trp Asn Asp Leu Asp
305         310         315         320
Val Asn Gln His Val Ser Asn Val Lys Tyr Ile Gly Trp Ile Leu Glu
325         330         335
Ser Met Pro Thr Glu Val Leu Glu Thr Gln Glu Leu Cys Ser Leu Ala
340         345         350
Leu Glu Tyr Arg Arg Glu Cys Gly Arg Asp Ser Val Leu Glu Ser Val
355         360         365
Thr Ala Met Asp Pro Ser Lys Val Gly Val Arg Ser Gln Tyr Gln His
370         375         380

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Leu Leu Arg Leu Glu Asp Gly Thr Ala Ile Val Asn Gly Ala Thr Glu
 385 390 395 400
 Trp Arg Pro Lys Asn Ala Gly Ala Asn Gly Ala Ile Ser Thr Gly Lys
 405 410 415
 Thr Ser Asn Gly Asn Ser Val Ser Met Asp Tyr Lys Asp His Asp Gly
 420 425 430
 Asp Tyr Lys Asp His Asp Ile Asp Tyr Lys Asp Asp Asp Asp Lys
 435 440 445

<210> SEQ ID NO 5
 <211> LENGTH: 447
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polypeptide

<400> SEQUENCE: 5

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30
 Pro Val Arg Gly Arg Ala Ser Ser Leu Ser Pro Ser Phe Lys Pro Lys
 35 40 45
 Ser Ile Pro Asn Gly Gly Phe Gln Val Lys Ala Asn Asp Ser Ala His
 50 55 60
 Pro Lys Ala Asn Gly Ser Ala Val Ser Leu Lys Ser Gly Ser Leu Asn
 65 70 75 80
 Thr Gln Glu Asp Thr Ser Ser Ser Pro Pro Pro Arg Thr Phe Leu His
 85 90 95
 Gln Leu Pro Asp Trp Ser Arg Leu Leu Thr Ala Ile Thr Thr Val Phe
 100 105 110
 Val Lys Ser Lys Arg Pro Asp Met His Asp Arg Lys Ser Lys Arg Pro
 115 120 125
 Asp Met Leu Val Asp Ser Phe Gly Leu Glu Ser Thr Val Gln Asp Gly
 130 135 140
 Leu Val Phe Arg Gln Ser Phe Ser Ile Arg Ser Tyr Glu Ile Gly Thr
 145 150 155 160
 Asp Arg Thr Ala Ser Ile Glu Thr Leu Met Asn His Leu Gln Glu Thr
 165 170 175
 Ser Leu Asn His Cys Lys Ser Thr Gly Ile Leu Leu Asp Gly Phe Gly
 180 185 190
 Arg Thr Ala Glu Met Cys Lys Arg Asp Leu Ile Trp Val Val Ile Lys
 195 200 205
 Met Gln Ile Lys Val Asn Arg Tyr Pro Ala Trp Gly Asp Thr Val Glu
 210 215 220
 Ile Asn Thr Arg Phe Ser Arg Leu Gly Lys Ile Gly Met Gly Arg Asp
 225 230 235 240
 Trp Leu Ile Ser Asp Cys Asn Thr Gly Glu Ile Leu Val Arg Ala Thr
 245 250 255
 Ser Ala Tyr Ala Met Met Asn Gln Lys Thr Arg Arg Leu Ser Lys Leu
 260 265 270
 Pro Tyr Glu Val His Gln Glu Ile Val Pro Leu Phe Val Asp Ser Pro
 275 280 285
 Val Ile Glu Asp Ser Asp Leu Lys Val His Lys Phe Lys Val Lys Thr

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Met Gln Ile Lys Val Asn Arg Tyr Pro Ala Trp Gly Asp Thr Val Glu
 210 215 220

Ile Asn Thr Arg Phe Ser Arg Leu Gly Lys Ile Gly Met Gly Arg Asp
 225 230 240

Trp Leu Ile Ser Asp Cys Asn Thr Gly Glu Ile Leu Val Arg Ala Thr
 245 250 255

Ser Ala Tyr Ala Met Met Asn Gln Lys Thr Arg Arg Leu Ser Lys Leu
 260 265 270

Pro Tyr Glu Val His Gln Glu Ile Val Pro Leu Phe Val Asp Ser Pro
 275 280 285

Val Ile Glu Asp Ser Asp Leu Lys Val His Lys Phe Lys Val Lys Thr
 290 295 300

Gly Asp Ser Ile Gln Lys Gly Leu Thr Pro Gly Trp Asn Asp Leu Asp
 305 310 315 320

Val Asn Gln His Val Ser Asn Val Lys Tyr Ile Gly Trp Ile Leu Glu
 325 330 335

Ser Met Pro Thr Glu Val Leu Glu Thr Gln Glu Leu Cys Ser Leu Ala
 340 345 350

Leu Glu Tyr Arg Arg Glu Cys Gly Arg Asp Ser Val Leu Glu Ser Val
 355 360 365

Thr Ala Met Asp Pro Ser Lys Val Gly Val Arg Ser Gln Tyr Gln His
 370 375 380

Leu Leu Arg Leu Glu Asp Gly Thr Ala Ile Val Asn Gly Ala Thr Glu
 385 390 395 400

Trp Arg Pro Lys Asn Ala Gly Ala Asn Gly Ala Ile Ser Thr Gly Lys
 405 410 415

Thr Ser Asn Gly Asn Ser Val Ser Met Asp Tyr Lys Asp His Asp Gly
 420 425 430

Asp Tyr Lys Asp His Asp Ile Asp Tyr Lys Asp Asp Asp Lys
 435 440 445

<210> SEQ ID NO 7
 <211> LENGTH: 447
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polypeptide

<400> SEQUENCE: 7

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15

Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30

Pro Val Arg Gly Arg Ala Ser Ser Leu Ser Pro Ser Phe Lys Pro Lys
 35 40 45

Ser Ile Pro Asn Gly Gly Phe Gln Val Lys Ala Asn Asp Ser Ala His
 50 55 60

Pro Lys Ala Asn Gly Ser Ala Val Ser Leu Lys Ser Gly Ser Leu Asn
 65 70 75 80

Thr Gln Glu Asp Thr Ser Ser Ser Pro Pro Pro Arg Thr Phe Leu His
 85 90 95

Gln Leu Pro Asp Trp Ser Arg Leu Leu Thr Ala Ile Thr Thr Val Phe
 100 105 110

Val Lys Ser Lys Arg Pro Asp Met His Asp Arg Lys Ser Lys Arg Pro
 115 120 125

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Asp Met Leu Val Asp Ser Phe Gly Leu Glu Ser Thr Val Gln Asp Gly
 130 135 140
 Leu Val Phe Arg Gln Ser Phe Ser Ile Arg Ser Tyr Glu Ile Gly Thr
 145 150 155 160
 Asp Arg Thr Ala Ser Ile Glu Thr Leu Met Asn Phe Leu Gln Glu Thr
 165 170 175
 Ser Leu Asn His Cys Lys Ser Thr Gly Ile Leu Leu Asp Gly Phe Gly
 180 185 190
 Arg Thr Leu Glu Met Cys Lys Arg Asp Leu Ile Trp Val Val Ile Lys
 195 200 205
 Met Gln Ile Lys Val Asn Arg Tyr Pro Ala Trp Gly Asp Thr Val Glu
 210 215 220
 Ile Asn Thr Arg Phe Ser Arg Leu Gly Lys Ile Gly Met Gly Arg Asp
 225 230 235 240
 Trp Leu Ile Ser Asp Cys Asn Thr Gly Glu Ile Leu Val Arg Ala Thr
 245 250 255
 Ser Ala Tyr Ala Met Met Asn Gln Lys Thr Arg Arg Leu Ser Lys Leu
 260 265 270
 Pro Tyr Glu Val His Gln Glu Ile Val Pro Leu Phe Val Asp Ser Pro
 275 280 285
 Val Ile Glu Asp Ser Asp Leu Lys Val His Lys Phe Lys Val Lys Thr
 290 295 300
 Gly Asp Ser Ile Gln Lys Gly Leu Thr Pro Gly Trp Asn Asp Leu Asp
 305 310 315 320
 Val Asn Gln His Val Ser Asn Val Lys Tyr Ile Gly Trp Ile Leu Glu
 325 330 335
 Ser Met Pro Thr Glu Val Leu Glu Thr Gln Glu Leu Cys Ser Leu Ala
 340 345 350
 Leu Glu Tyr Arg Arg Glu Cys Gly Arg Asp Ser Val Leu Glu Ser Val
 355 360 365
 Thr Ala Met Asp Pro Ser Lys Val Gly Val Arg Ser Gln Tyr Gln His
 370 375 380
 Leu Leu Arg Leu Glu Asp Gly Thr Ala Ile Val Asn Gly Ala Thr Glu
 385 390 395 400
 Trp Arg Pro Lys Asn Ala Gly Ala Asn Gly Ala Ile Ser Thr Gly Lys
 405 410 415
 Thr Ser Asn Gly Asn Ser Val Ser Met Asp Tyr Lys Asp His Asp Gly
 420 425 430
 Asp Tyr Lys Asp His Asp Ile Asp Tyr Lys Asp Asp Asp Lys
 435 440 445

<210> SEQ ID NO 8

<211> LENGTH: 447

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 8

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30
 Pro Val Arg Gly Arg Ala Ser Ser Leu Ser Pro Ser Phe Lys Pro Lys

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35					40					45					
Ser	Ile	Pro	Asn	Gly	Gly	Phe	Gln	Val	Lys	Ala	Asn	Asp	Ser	Ala	His
50					55					60					
Pro	Lys	Ala	Asn	Gly	Ser	Ala	Val	Ser	Leu	Lys	Ser	Gly	Ser	Leu	Asn
65					70					75					80
Thr	Gln	Glu	Asp	Thr	Ser	Ser	Ser	Pro	Pro	Pro	Arg	Thr	Phe	Leu	His
				85					90					95	
Gln	Leu	Pro	Asp	Trp	Ser	Arg	Leu	Leu	Thr	Ala	Ile	Thr	Thr	Val	Phe
			100					105						110	
Val	Lys	Ser	Lys	Arg	Pro	Asp	Met	His	Asp	Arg	Lys	Ser	Lys	Arg	Pro
			115				120					125			
Asp	Met	Leu	Val	Asp	Ser	Phe	Gly	Leu	Glu	Ser	Thr	Val	Gln	Asp	Gly
	130					135					140				
Leu	Val	Phe	Arg	Gln	Ser	Phe	Ser	Ile	Arg	Ser	Tyr	Glu	Ile	Gly	Thr
145						150					155				160
Asp	Arg	Thr	Ala	Ser	Ile	Glu	Thr	Leu	Met	Asn	His	Leu	Gln	Glu	Thr
			165						170					175	
Ser	Leu	Asn	His	Cys	Lys	Ser	Thr	Gly	Ile	Leu	Leu	Asp	Gly	Phe	Gly
			180					185						190	
Arg	Thr	Leu	Glu	Met	Cys	Lys	Arg	Asp	Leu	Ile	Trp	Val	Val	Ile	Lys
			195				200					205			
Met	Gln	Ile	Lys	Val	Asn	Arg	Tyr	Pro	Ala	Trp	Gly	Asp	Thr	Val	Glu
	210					215					220				
Ile	Asn	Thr	Arg	Phe	Ser	Arg	Leu	Gly	Lys	Ile	Gly	Met	Gly	Arg	Asp
225						230					235				240
Trp	Leu	Ile	Ser	Asp	Cys	Asn	Thr	Gly	Glu	Ile	Leu	Val	Arg	Ala	Thr
			245						250					255	
Ser	Ala	Tyr	Ala	Met	Met	Asn	Gln	Lys	Thr	Arg	Arg	Leu	Ser	Lys	Leu
			260					265					270		
Pro	Tyr	Glu	Val	His	Gln	Glu	Ile	Val	Pro	Leu	Phe	Val	Asp	Ser	Pro
		275					280					285			
Val	Ile	Glu	Asp	Ser	Asp	Leu	Lys	Val	His	Lys	Phe	Lys	Val	Lys	Thr
	290					295					300				
Gly	Asp	Ser	Ile	Gln	Lys	Gly	Leu	Thr	Pro	Gly	Trp	Asn	Asp	Leu	Asp
305						310					315				320
Val	Asn	Gln	His	Val	Ser	Asn	Val	Lys	Tyr	Ile	Gly	Trp	Ile	Leu	Glu
			325						330					335	
Ser	Met	Pro	Thr	Glu	Val	Leu	Glu	Thr	Gln	Glu	Leu	Cys	Ser	Leu	Ala
			340					345					350		
Leu	Glu	Tyr	Arg	Arg	Glu	Cys	Gly	Arg	Asp	Ser	Val	Leu	Glu	Ser	Val
		355					360					365			
Thr	Ala	Met	Asp	Pro	Ser	Lys	Val	Gly	Val	Arg	Ser	Gln	Tyr	Gln	His
		370				375					380				
Leu	Leu	Arg	Leu	Glu	Asp	Gly	Thr	Ala	Ile	Val	Asn	Gly	Ala	Thr	Glu
385						390					395				400
Trp	Arg	Pro	Lys	Asn	Ala	Gly	Ala	Asn	Gly	Ala	Ile	Ser	Thr	Gly	Lys
			405						410					415	
Thr	Ser	Asn	Gly	Asn	Ser	Val	Ser	Met	Asp	Tyr	Lys	Asp	His	Asp	Gly
			420					425					430		
Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys	
		435					440					445			

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<211> LENGTH: 6075
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide

<400> SEQUENCE: 9
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ttcgtccaga gacggcaagt cgtccatcct ctgcgtgtgt ggcgcgacgc tgcagcagtc    180
cctctgcagc agatgagcgt gactttggcc atttcacgca ctcgagtgtg cacaatccat    240
ttttcttaaa gcaaatgact gctgattgac cagatactgt aacgctgatt tcgctccaga    300
tcgcacagat agcgaccatg ttgctgcgtc tgaaaaatctg gattccgaat tcgaccctgg    360
cgctccatcc atgcaacaga tggcgacact tgttaacaatt cctgtcacc c atcggcatgg    420
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ctctcgcagg caaagccggg cacttgccct ccagattgcc aataaactca attatggcct    660
ctgtcatgcc atccatgggt ctgatgaatg gtcacgctcg tgtcctgacc gttccccagc    720
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gacggcttcc cggcgtgca tgcaacaccg atgatgcttc gacccccga agctccttcg    900
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cgattgcaaa gacattatag cgagctacca aagccatatt caaacaccta gatcactacc   1020
actttacac aggccactcg agcttgtgat cgcactccgc taagggggcg cctcttctc   1080
ttcgtttcag tcacaacccg caaactctag aatatcaatg atcgagcagg acggcctcca   1140
cgccggctcc cccgcgcctc ggggtggagcg cctgttcggc tacgactggg cccagcagac   1200
catcggctgc tccgacgcgc ccgtgttccg cctgtccgcc cagggccgcc ccgtgctgtt   1260
cgtgaagacc gacctgtccg gcgcccgtga cgagctgcag gacgaggccg cccgcctgtc   1320
ctggctggcc accaccggcg tgcccgtgcg ccgcgtgctg gacgtggtga ccgaggccgg   1380
ccgcgactgg ctgctgctgg gcgaggtgcc cggccaggac ctgctgtcct cccacctggc   1440
ccccgccgag aaggtgtcca tcattggcca cgccatgcgc cgcctgcaca ccctggacct   1500
cgccacctgc cccttcgacc accaggccaa gcaccgcac gagcgcgccc gcaccgcgat   1560
ggaggccggc ctggtggacc aggaagacct ggacgaggag caccaggggc tggccccgcg   1620
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gacgacaagt gactcgaggc agcagcagct cggatagtat cgacacactc tggacgctgg 4980
tcgtgtgatg gactgttgcc gccacacttg ctgccttgac ctgtgaatat ccctgccgct 5040
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catcccaacc gcaacttatt tacgctgtcc tgctatccct cagcgtgct cctgctcctg 5220
ctcactgccc ctgcacagc cttggtttg gctccgctg tattctcctg gtactgcaac 5280
ctgtaaacca gcaactgcaat gctgatgac gggaaagtagt gggatgggaa cacaaatgga 5340
aagctgtata gggataacag ggtaatgagc tccagcgcga tgccacgccc tttgatggct 5400
tcaagtacga ttaagggtgtt ggattgtgtg tttgttgctg agtgtgcatg gtttagaata 5460
atacacttga tttcttgctc acggcaatct cggcttgctc gcaggttcaa cccatttctg 5520
gagtctcagg tcagccgcgc aatgaccagc cgctacttca aggacttgca cgacaacgcc 5580
gaggtgagct atgttttaga cttgattgga aattgtcgtc gacgcatatt cgcgctccgc 5640
gacagcacc c aagcaaaatg tcaagtgcgt tccgatttgc gtccgcaggt cgatgttgtg 5700
atcgctcggcg ccggatccgc cggctctgtc tgcgcttacg agctgaccaa gcaccctgac 5760
gtccgggtac gcgagctgag attcgattag acataaattg aagattaaac ccgtagaaaa 5820
atgtgatggt cgcgaaaactg tgctcgattg caagaaattg atcgtcctcc actccgcagg 5880
tcgccatcat cgagcagggc gttgctcccg gcggcggcgc ctggctgggg ggacagctgt 5940
tctcggccat gtgtgtacgt agaaggatga atttcagctg gttttcgttg cacagctgtt 6000
tgtgcatgat ttgtttcaga ctattgttga atgttttag atttcttagg atgcatgatt 6060
tgtctgcatg cgact 6075

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<210> SEQ ID NO 10

<211> LENGTH: 444

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 10

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Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1             5             10             15
Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20            25            30
Pro Val Arg Gly Arg Ala Ser Ser Leu Ser Pro Ser Leu Lys Pro
 35            40            45
Lys Ser Ile Pro Asn Gly Gly Phe Gln Val Lys Ala Asn Ala Ser Ala
 50            55            60
His Pro Lys Ala Asn Gly Ser Ala Val Thr Leu Lys Ser Gly Ser Leu

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65	70	75	80
Asn Thr Gln Glu Asp Thr Leu Ser Ser Ser Pro Pro Pro Arg Ala Phe	85	90	95
Phe Asn Gln Leu Pro Asp Trp Ser Met Leu Leu Thr Ala Ile Thr Thr	100	105	110
Val Phe Val Ala Pro Glu Lys Arg Trp Thr Met Phe Asp Arg Lys Ser	115	120	125
Lys Arg Pro Asn Met Leu Met Asp Ser Phe Gly Leu Glu Arg Val Val	130	135	140
Gln Asp Gly Leu Val Phe Arg Gln Ser Phe Ser Ile Arg Ser Tyr Glu	145	150	155
Ile Cys Ala Asp Arg Thr Ala Ser Ile Glu Thr Val Met Asn His Val	165	170	175
Gln Glu Thr Ser Leu Asn Gln Cys Lys Ser Ile Gly Leu Leu Asp Asp	180	185	190
Gly Phe Gly Arg Ser Pro Glu Met Cys Lys Arg Asp Leu Ile Trp Val	195	200	205
Val Thr Arg Met Lys Ile Met Val Asn Arg Tyr Pro Thr Trp Gly Asp	210	215	220
Thr Ile Glu Val Ser Thr Trp Leu Ser Gln Ser Gly Lys Ile Gly Met	225	230	235
Gly Arg Asp Trp Leu Ile Ser Asp Cys Asn Thr Gly Glu Ile Leu Val	245	250	255
Arg Ala Thr Ser Val Tyr Ala Met Met Asn Gln Lys Thr Arg Arg Phe	260	265	270
Ser Lys Leu Pro His Glu Val Arg Gln Glu Phe Ala Pro His Phe Leu	275	280	285
Asp Ser Pro Pro Ala Ile Glu Asp Asn Asp Gly Lys Leu Gln Lys Phe	290	295	300
Asp Val Lys Thr Gly Asp Ser Ile Arg Lys Gly Leu Thr Pro Gly Trp	305	310	315
Tyr Asp Leu Asp Val Asn Gln His Val Ser Asn Val Lys Tyr Ile Gly	325	330	335
Trp Ile Leu Glu Ser Met Pro Thr Glu Val Leu Glu Thr Gln Glu Leu	340	345	350
Cys Ser Leu Thr Leu Glu Tyr Arg Arg Glu Cys Gly Arg Asp Ser Val	355	360	365
Leu Glu Ser Val Thr Ser Met Asp Pro Ser Lys Val Gly Asp Arg Phe	370	375	380
Gln Tyr Arg His Leu Leu Arg Leu Glu Asp Gly Ala Asp Ile Met Lys	385	390	395
Gly Arg Thr Glu Trp Arg Pro Lys Asn Ala Gly Thr Asn Gly Ala Ile	405	410	415
Ser Thr Gly Lys Thr Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys	420	425	430
Asp His Asp Ile Asp Tyr Lys Asp Asp Asp Asp Lys	435	440	

<210> SEQ ID NO 11

<211> LENGTH: 447

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

-continued

<400> SEQUENCE: 11

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30
 Pro Val Arg Gly Arg Ala Ser Ser Leu Ser Pro Ser Phe Lys Pro Lys
 35 40 45
 Ser Ile Pro Asn Gly Gly Phe Gln Val Lys Ala Asn Asp Ser Ala His
 50 55 60
 Pro Lys Ala Asn Gly Ser Ala Val Ser Leu Lys Ser Gly Ser Leu Asn
 65 70 75 80
 Thr Gln Glu Asp Thr Ser Ser Ser Pro Pro Pro Arg Thr Phe Leu His
 85 90 95
 Gln Leu Pro Asp Trp Ser Arg Leu Leu Thr Ala Ile Thr Thr Val Phe
 100 105 110
 Val Lys Ser Lys Arg Pro Asp Met His Asp Arg Lys Ser Lys Arg Pro
 115 120 125
 Asp Met Leu Val Asp Ser Phe Gly Leu Glu Ser Thr Val Gln Asp Gly
 130 135 140
 Leu Val Phe Arg Gln Ser Phe Ser Ile Arg Ser Tyr Glu Ile Gly Thr
 145 150 155 160
 Asp Arg Thr Ala Ser Ile Glu Thr Leu Met Asn His Leu Gln Glu Thr
 165 170 175
 Ser Leu Asn His Cys Lys Ser Thr Gly Ile Leu Leu Asp Gly Phe Gly
 180 185 190
 Arg Thr Leu Glu Met Cys Lys Arg Asp Leu Ile Trp Val Val Ile Lys
 195 200 205
 Met Gln Ile Lys Val Asn Arg Tyr Pro Ala Trp Gly Asp Thr Val Glu
 210 215 220
 Ile Asn Thr Arg Phe Ser Arg Leu Gly Lys Ile Gly Met Gly Arg Asp
 225 230 235 240
 Trp Leu Ile Ser Asp Cys Asn Thr Gly Glu Ile Leu Val Arg Ala Thr
 245 250 255
 Ser Ala Tyr Ala Met Met Asn Gln Lys Thr Arg Arg Leu Ser Lys Leu
 260 265 270
 Pro Tyr Glu Val His Gln Glu Ile Val Pro Leu Phe Val Asp Ser Pro
 275 280 285
 Val Ile Glu Asp Ser Asp Leu Lys Val His Lys Phe Lys Val Lys Thr
 290 295 300
 Gly Asp Ser Ile Gln Lys Gly Leu Thr Pro Gly Trp Asn Asp Leu Asp
 305 310 315 320
 Val Asn Gln His Val Ser Asn Val Lys Tyr Ile Gly Trp Ile Leu Glu
 325 330 335
 Ser Met Pro Thr Glu Val Leu Glu Thr Gln Glu Leu Cys Ser Leu Ala
 340 345 350
 Leu Glu Tyr Arg Arg Glu Cys Gly Arg Asp Ser Val Leu Glu Ser Val
 355 360 365
 Thr Ala Met Asp Pro Ser Lys Val Gly Val Arg Ser Gln Tyr Gln His
 370 375 380
 Leu Leu Arg Leu Glu Asp Gly Thr Ala Ile Val Asn Gly Ala Thr Glu
 385 390 395 400
 Trp Arg Pro Lys Asn Ala Gly Ala Asn Gly Ala Ile Ser Thr Gly Lys

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405 410 415
 Thr Ser Asn Gly Asn Ser Val Ser Met Asp Tyr Lys Asp His Asp Gly
 420 425 430
 Asp Tyr Lys Asp His Asp Ile Asp Tyr Lys Asp Asp Asp Asp Lys
 435 440 445

<210> SEQ ID NO 12
 <211> LENGTH: 427
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polypeptide

<400> SEQUENCE: 12

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30
 Pro Val Arg Ala Ala Ile Asn Ser Arg Ala His Pro Lys Ala Asn Gly
 35 40 45
 Ser Ala Val Ser Leu Lys Ser Gly Ser Leu Asn Thr Gln Glu Asp Thr
 50 55 60
 Ser Ser Ser Pro Pro Pro Arg Thr Phe Leu His Gln Leu Pro Asp Trp
 65 70 75 80
 Ser Arg Leu Leu Thr Ala Ile Thr Thr Val Phe Val Lys Ser Lys Arg
 85 90 95
 Pro Asp Met His Asp Arg Lys Ser Lys Arg Pro Asp Met Leu Met Asp
 100 105 110
 Ser Phe Gly Leu Glu Ser Ile Val Gln Glu Gly Leu Glu Phe Arg Gln
 115 120 125
 Ser Phe Ser Ile Arg Ser Tyr Glu Ile Gly Thr Asp Arg Thr Ala Ser
 130 135 140
 Ile Glu Thr Leu Met Asn Tyr Leu Gln Glu Thr Ser Leu Asn His Cys
 145 150 155 160
 Lys Ser Thr Gly Ile Leu Leu Asp Gly Phe Gly Arg Thr Pro Glu Met
 165 170 175
 Cys Lys Arg Asp Leu Ile Trp Val Val Thr Lys Met Lys Ile Lys Val
 180 185 190
 Asn Arg Tyr Pro Ala Trp Gly Asp Thr Val Glu Ile Asn Thr Trp Phe
 195 200 205
 Ser Arg Leu Gly Lys Ile Gly Lys Gly Arg Asp Trp Leu Ile Ser Asp
 210 215 220
 Cys Asn Thr Gly Glu Ile Leu Ile Arg Ala Thr Ser Ala Tyr Ala Thr
 225 230 235 240
 Met Asn Gln Lys Thr Arg Arg Leu Ser Lys Leu Pro Tyr Glu Val His
 245 250 255
 Gln Glu Ile Ala Pro Leu Phe Val Asp Ser Pro Pro Val Ile Glu Asp
 260 265 270
 Asn Asp Leu Lys Leu His Lys Phe Glu Val Lys Thr Gly Asp Ser Ile
 275 280 285
 His Lys Gly Leu Thr Pro Gly Trp Asn Asp Leu Asp Val Asn Gln His
 290 295 300
 Val Ser Asn Val Lys Tyr Ile Gly Trp Ile Leu Glu Ser Met Pro Thr
 305 310 315 320

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Phe Ala Pro His Phe Leu Asp Ser Pro Pro Ala Ile Glu Asp Asn Asp
 275 280 285

Gly Lys Leu Gln Lys Phe Asp Val Lys Thr Gly Asp Ser Ile Arg Lys
 290 295 300

Gly Leu Thr Pro Gly Trp Tyr Asp Leu Asp Val Asn Gln His Val Ser
 305 310 315 320

Asn Val Lys Tyr Ile Gly Trp Ile Leu Glu Ser Met Pro Thr Glu Val
 325 330 335

Leu Glu Thr Gln Glu Leu Cys Ser Leu Thr Leu Glu Tyr Arg Arg Glu
 340 345 350

Cys Gly Arg Asp Ser Val Leu Glu Ser Val Thr Ser Met Asp Pro Ser
 355 360 365

Lys Val Gly Asp Arg Phe Gln Tyr Arg His Leu Leu Arg Leu Glu Asp
 370 375 380

Gly Ala Asp Ile Met Lys Gly Arg Thr Glu Trp Arg Pro Lys Asn Ala
 385 390 395 400

Gly Thr Asn Gly Ala Ile Ser Thr Gly Lys Thr
 405 410

<210> SEQ ID NO 14
 <211> LENGTH: 416
 <212> TYPE: PRT
 <213> ORGANISM: Cuphea avigera

<400> SEQUENCE: 14

Met Val Ala Ala Ala Ala Ser Ser Ala Phe Phe Ser Val Pro Val Pro
 1 5 10 15

Gly Thr Ser Pro Lys Pro Gly Lys Phe Arg Ile Trp Pro Ser Ser Leu
 20 25 30

Ser Pro Ser Phe Lys Pro Lys Pro Ile Pro Asn Gly Gly Leu Gln Val
 35 40 45

Lys Ala Asn Ser Arg Ala His Pro Lys Ala Asn Gly Ser Ala Val Ser
 50 55 60

Leu Lys Ser Gly Ser Leu Asn Thr Gln Glu Asp Thr Ser Ser Ser Pro
 65 70 75 80

Pro Pro Arg Thr Phe Leu His Gln Leu Pro Asp Trp Ser Arg Leu Leu
 85 90 95

Thr Ala Ile Thr Thr Val Phe Val Lys Ser Lys Arg Pro Asp Met His
 100 105 110

Asp Arg Lys Ser Lys Arg Pro Asp Met Leu Met Asp Ser Phe Gly Leu
 115 120 125

Glu Ser Ile Val Gln Glu Gly Leu Glu Phe Arg Gln Ser Phe Ser Ile
 130 135 140

Arg Ser Tyr Glu Ile Gly Thr Asp Arg Thr Ala Ser Ile Glu Thr Leu
 145 150 155 160

Met Asn Tyr Leu Gln Glu Thr Ser Leu Asn His Cys Lys Ser Thr Gly
 165 170 175

Ile Leu Leu Asp Gly Phe Gly Arg Thr Pro Glu Met Cys Lys Arg Asp
 180 185 190

Leu Ile Trp Val Val Thr Lys Met Lys Ile Lys Val Asn Arg Tyr Pro
 195 200 205

Ala Trp Gly Asp Thr Val Glu Ile Asn Thr Trp Phe Ser Arg Leu Gly
 210 215 220

Lys Ile Gly Lys Gly Arg Asp Trp Leu Ile Ser Asp Cys Asn Thr Gly

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225	230	235	240
Glu Ile Leu Ile Arg Ala Thr Ser Ala Tyr Ala Thr Met Asn Gln Lys	245	250	255
Thr Arg Arg Leu Ser Lys Leu Pro Tyr Glu Val His Gln Glu Ile Ala	260	265	270
Pro Leu Phe Val Asp Ser Pro Pro Val Ile Glu Asp Asn Asp Leu Lys	275	280	285
Leu His Lys Phe Glu Val Lys Thr Gly Asp Ser Ile His Lys Gly Leu	290	295	300
Thr Pro Gly Trp Asn Asp Leu Asp Val Asn Gln His Val Ser Asn Val	305	310	315
Lys Tyr Ile Gly Trp Ile Leu Glu Ser Met Pro Thr Glu Val Leu Glu	325	330	335
Thr Gln Glu Leu Cys Ser Leu Ala Leu Glu Tyr Arg Arg Glu Cys Gly	340	345	350
Arg Asp Ser Val Leu Glu Ser Val Thr Ala Met Asp Pro Thr Lys Val	355	360	365
Gly Gly Arg Ser Gln Tyr Gln His Leu Leu Arg Leu Glu Asp Gly Thr	370	375	380
Asp Ile Val Lys Cys Arg Thr Glu Trp Arg Pro Lys Asn Pro Gly Ala	385	390	395
Asn Gly Ala Ile Ser Thr Gly Lys Thr Ser Asn Gly Asn Ser Val Ser	405	410	415

<210> SEQ ID NO 15

<211> LENGTH: 391

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 15

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp	1	5	10	15
Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu	20	25	30	
Pro Val Arg Gly Arg Ala Ile Pro Pro Arg Ile Ile Val Val Ser Ser	35	40	45	
Ser Ser Ser Lys Val Asn Pro Leu Lys Thr Glu Ala Val Val Ser Ser	50	55	60	
Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu	65	70	75	80
Ser Tyr Lys Glu Lys Phe Ile Val Arg Cys Tyr Glu Val Gly Ile Asn	85	90	95	
Lys Thr Ala Thr Val Glu Thr Ile Ala Asn Leu Leu Gln Glu Val Gly	100	105	110	
Cys Asn His Ala Gln Ser Val Gly Tyr Ser Thr Gly Gly Phe Ser Thr	115	120	125	
Thr Pro Thr Met Arg Lys Leu Arg Leu Ile Trp Val Thr Ala Arg Met	130	135	140	
His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu Ile	145	150	155	160
Glu Ser Trp Gly Gln Gly Glu Gly Lys Ile Gly Thr Arg Arg Asp Trp	165	170	175	

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Ile Leu Arg Asp Tyr Ala Thr Gly Gln Val Ile Gly Arg Ala Thr Ser
      180                      185                      190
Lys Trp Val Met Met Asn Gln Asp Thr Arg Arg Leu Gln Lys Val Asp
      195                      200                      205
Val Asp Val Arg Asp Glu Tyr Leu Val His Cys Pro Arg Glu Leu Arg
      210                      215                      220
Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys
      225                      230                      235                      240
Leu Glu Asp Pro Ser Gln Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg
      245                      250                      255
Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile Gly
      260                      265                      270
Trp Val Leu Glu Ser Met Pro Gln Glu Ile Ile Asp Thr His Glu Leu
      275                      280                      285
Gln Thr Ile Thr Leu Asp Tyr Arg Arg Glu Cys Gln His Asp Asp Val
      290                      295                      300
Val Asp Ser Leu Thr Ser Pro Glu Pro Ser Glu Asp Ala Glu Ala Val
      305                      310                      315                      320
Phe Asn His Asn Gly Thr Asn Gly Ser Ala Asn Val Ser Ala Asn Asp
      325                      330                      335
His Gly Cys Arg Asn Phe Leu His Leu Leu Arg Leu Ser Gly Asn Gly
      340                      345                      350
Leu Glu Ile Asn Arg Gly Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
      355                      360                      365
Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
      370                      375                      380
Tyr Lys Asp Asp Asp Asp Lys
      385                      390

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<210> SEQ ID NO 16
<211> LENGTH: 391
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

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<400> SEQUENCE: 16

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Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
  1      5      10
Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
  20     25     30
Pro Val Arg Gly Arg Ala Ile Pro Pro Arg Ile Ile Val Val Ser Ser
  35     40     45
Ser Ser Ser Lys Val Asn Pro Leu Lys Thr Glu Ala Val Val Ser Ser
  50     55     60
Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu
  65     70     75     80
Ser Tyr Lys Glu Lys Phe Ile Val Arg Cys Tyr Glu Val Gly Ile Asn
  85     90     95
Lys Thr Ala Thr Val Glu Thr Ile Ala Asn Leu Leu Gln Glu Val Gly
  100    105    110
Cys Asn His Ala Gln Ser Val Gly Tyr Ser Thr Gly Gly Phe Ala Thr
  115    120    125
Thr Pro Thr Met Arg Lys Leu Arg Leu Ile Trp Val Thr Ala Arg Met
  130    135    140

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His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu Ile
 145 150 155 160
 Glu Ser Trp Gly Gln Gly Glu Gly Lys Ile Gly Thr Arg Arg Asp Trp
 165 170 175
 Ile Leu Arg Asp Tyr Ala Thr Gly Gln Val Ile Gly Arg Ala Thr Ser
 180 185 190
 Lys Trp Val Met Met Asn Gln Asp Thr Arg Arg Leu Gln Lys Val Asp
 195 200 205
 Ala Asp Val Arg Asp Glu Tyr Leu Val His Cys Pro Arg Glu Leu Arg
 210 215 220
 Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys
 225 230 235 240
 Leu Glu Asp Pro Ser Gln Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg
 245 250 255
 Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile Gly
 260 265 270
 Trp Val Leu Glu Ser Met Pro Gln Glu Ile Ile Asp Thr His Glu Leu
 275 280 285
 Gln Thr Ile Thr Leu Asp Tyr Arg Arg Glu Cys Gln His Asp Asp Val
 290 295 300
 Val Asp Ser Leu Thr Ser Pro Glu Pro Ser Glu Asp Ala Glu Ala Val
 305 310 315 320
 Phe Asn His Asn Gly Thr Asn Gly Ser Ala Asn Val Ser Ala Asn Asp
 325 330 335
 His Gly Cys Arg Asn Phe Leu His Leu Leu Arg Leu Ser Gly Asn Gly
 340 345 350
 Leu Glu Ile Asn Arg Gly Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
 355 360 365
 Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
 370 375 380
 Tyr Lys Asp Asp Asp Asp Lys
 385 390

<210> SEQ ID NO 17

<211> LENGTH: 391

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 17

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30
 Pro Val Arg Gly Arg Ala Ile Pro Pro Arg Ile Ile Val Val Ser Ser
 35 40 45
 Ser Ser Ser Lys Val Asn Pro Leu Lys Thr Glu Ala Val Val Ser Ser
 50 55 60
 Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu
 65 70 75 80
 Ser Tyr Lys Glu Lys Phe Ile Val Arg Cys Tyr Glu Val Gly Ile Asn
 85 90 95
 Lys Thr Ala Thr Val Glu Thr Ile Ala Asn Leu Leu Gln Glu Val Gly

-continued

	100						105								110
Cys	Asn	His	Ala	Gln	Ser	Val	Gly	Tyr	Ser	Thr	Gly	Gly	Phe	Val	Thr
	115						120					125			
Thr	Pro	Thr	Met	Arg	Lys	Leu	Arg	Leu	Ile	Trp	Val	Thr	Ala	Arg	Met
	130						135				140				
His	Ile	Glu	Ile	Tyr	Lys	Tyr	Pro	Ala	Trp	Ser	Asp	Val	Val	Glu	Ile
145					150					155				160	
Glu	Ser	Trp	Gly	Gln	Gly	Glu	Gly	Lys	Ile	Gly	Thr	Arg	Arg	Asp	Trp
				165					170					175	
Ile	Leu	Arg	Asp	Tyr	Ala	Thr	Gly	Gln	Val	Ile	Gly	Arg	Ala	Thr	Ser
			180					185					190		
Lys	Trp	Val	Met	Met	Asn	Gln	Asp	Thr	Arg	Arg	Leu	Gln	Lys	Val	Asp
		195					200					205			
Ala	Asp	Val	Arg	Asp	Glu	Tyr	Leu	Val	His	Cys	Pro	Arg	Glu	Leu	Arg
	210					215					220				
Leu	Ala	Phe	Pro	Glu	Glu	Asn	Asn	Ser	Ser	Leu	Lys	Lys	Ile	Ser	Lys
225					230					235				240	
Leu	Glu	Asp	Pro	Ser	Gln	Tyr	Ser	Lys	Leu	Gly	Leu	Val	Pro	Arg	Arg
				245					250					255	
Ala	Asp	Leu	Asp	Met	Asn	Gln	His	Val	Asn	Asn	Val	Thr	Tyr	Ile	Gly
		260						265						270	
Trp	Val	Leu	Glu	Ser	Met	Pro	Gln	Glu	Ile	Ile	Asp	Thr	His	Glu	Leu
		275					280					285			
Gln	Thr	Ile	Thr	Leu	Asp	Tyr	Arg	Arg	Glu	Cys	Gln	His	Asp	Asp	Val
	290					295					300				
Val	Asp	Ser	Leu	Thr	Ser	Pro	Glu	Pro	Ser	Glu	Asp	Ala	Glu	Ala	Val
305					310					315					320
Phe	Asn	His	Asn	Gly	Thr	Asn	Gly	Ser	Ala	Asn	Val	Ser	Ala	Asn	Asp
				325					330					335	
His	Gly	Cys	Arg	Asn	Phe	Leu	His	Leu	Leu	Arg	Leu	Ser	Gly	Asn	Gly
		340						345						350	
Leu	Glu	Ile	Asn	Arg	Gly	Arg	Thr	Glu	Trp	Arg	Lys	Lys	Pro	Thr	Arg
		355					360					365			
Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp
	370					375					380				
Tyr	Lys	Asp	Asp	Asp	Asp	Lys									
385					390										

<210> SEQ ID NO 18
 <211> LENGTH: 391
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 18

Met	Ala	Thr	Ala	Ser	Thr	Phe	Ser	Ala	Phe	Asn	Ala	Arg	Cys	Gly	Asp
1				5						10				15	
Leu	Arg	Arg	Ser	Ala	Gly	Ser	Gly	Pro	Arg	Arg	Pro	Ala	Arg	Pro	Leu
			20					25					30		
Pro	Val	Arg	Gly	Arg	Ala	Ile	Pro	Pro	Arg	Ile	Ile	Val	Val	Ser	Ser
		35					40					45			
Ser	Ser	Ser	Lys	Val	Asn	Pro	Leu	Lys	Thr	Glu	Ala	Val	Val	Ser	Ser
	50					55					60				

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Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu
 65 70 75 80
 Ser Tyr Lys Glu Lys Phe Ile Val Arg Cys Tyr Glu Val Gly Ile Asn
 85 90 95
 Lys Thr Ala Thr Val Glu Thr Ile Ala Asn Leu Leu Gln Glu Val Ala
 100 105 110
 Cys Asn His Ala Gln Ser Val Gly Tyr Ser Thr Gly Gly Phe Ser Thr
 115 120 125
 Thr Pro Thr Met Arg Lys Leu Arg Leu Ile Trp Val Thr Ala Arg Met
 130 135 140
 His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu Ile
 145 150 155 160
 Glu Ser Trp Gly Gln Gly Glu Gly Lys Ile Gly Thr Arg Arg Asp Trp
 165 170 175
 Ile Leu Arg Asp Tyr Ala Thr Gly Gln Val Ile Gly Arg Ala Thr Ser
 180 185 190
 Lys Trp Val Met Met Asn Gln Asp Thr Arg Arg Leu Gln Lys Val Asp
 195 200 205
 Val Asp Val Arg Asp Glu Tyr Leu Val His Cys Pro Arg Glu Leu Arg
 210 215 220
 Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys
 225 230 235 240
 Leu Glu Asp Pro Ser Gln Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg
 245 250 255
 Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile Gly
 260 265 270
 Trp Val Leu Glu Ser Met Pro Gln Glu Ile Ile Asp Thr His Glu Leu
 275 280 285
 Gln Thr Ile Thr Leu Asp Tyr Arg Arg Glu Cys Gln His Asp Asp Val
 290 295 300
 Val Asp Ser Leu Thr Ser Pro Glu Pro Ser Glu Asp Ala Glu Ala Val
 305 310 315 320
 Phe Asn His Asn Gly Thr Asn Gly Ser Ala Asn Val Ser Ala Asn Asp
 325 330 335
 His Gly Cys Arg Asn Phe Leu His Leu Leu Arg Leu Ser Gly Asn Gly
 340 345 350
 Leu Glu Ile Asn Arg Gly Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
 355 360 365
 Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
 370 375 380
 Tyr Lys Asp Asp Asp Lys
 385 390

<210> SEQ ID NO 19

<211> LENGTH: 391

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 19

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30

-continued

Pro Val Arg Gly Arg Ala Ile Pro Pro Arg Ile Ile Val Val Ser Ser
 35 40 45
 Ser Ser Ser Lys Val Asn Pro Leu Lys Thr Glu Ala Val Val Ser Ser
 50 55 60
 Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu
 65 70 75 80
 Ser Tyr Lys Glu Lys Phe Ile Val Arg Cys Tyr Glu Val Gly Ile Asn
 85 90 95
 Lys Thr Ala Thr Val Glu Thr Ile Ala Asn Leu Leu Gln Glu Val Thr
 100 105 110
 Cys Asn His Ala Gln Ser Val Gly Tyr Ser Thr Gly Gly Phe Ser Thr
 115 120 125
 Thr Pro Thr Met Arg Lys Leu Arg Leu Ile Trp Val Thr Ala Arg Met
 130 135 140
 His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu Ile
 145 150 155 160
 Glu Ser Trp Gly Gln Gly Glu Gly Lys Ile Gly Thr Arg Arg Asp Trp
 165 170 175
 Ile Leu Arg Asp Tyr Ala Thr Gly Gln Val Ile Gly Arg Ala Thr Ser
 180 185 190
 Lys Trp Val Met Met Asn Gln Asp Thr Arg Arg Leu Gln Lys Val Asp
 195 200 205
 Val Asp Val Arg Asp Glu Tyr Leu Val His Cys Pro Arg Glu Leu Arg
 210 215 220
 Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys
 225 230 235 240
 Leu Glu Asp Pro Ser Gln Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg
 245 250 255
 Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile Gly
 260 265 270
 Trp Val Leu Glu Ser Met Pro Gln Glu Ile Ile Asp Thr His Glu Leu
 275 280 285
 Gln Thr Ile Thr Leu Asp Tyr Arg Arg Glu Cys Gln His Asp Asp Val
 290 295 300
 Val Asp Ser Leu Thr Ser Pro Glu Pro Ser Glu Asp Ala Glu Ala Val
 305 310 315 320
 Phe Asn His Asn Gly Thr Asn Gly Ser Ala Asn Val Ser Ala Asn Asp
 325 330 335
 His Gly Cys Arg Asn Phe Leu His Leu Leu Arg Leu Ser Gly Asn Gly
 340 345 350
 Leu Glu Ile Asn Arg Gly Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
 355 360 365
 Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
 370 375 380
 Tyr Lys Asp Asp Asp Asp Lys
 385 390

<210> SEQ ID NO 20

<211> LENGTH: 391

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

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<400> SEQUENCE: 20

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30
 Pro Val Arg Gly Arg Ala Ile Pro Pro Arg Ile Ile Val Val Ser Ser
 35 40 45
 Ser Ser Ser Lys Val Asn Pro Leu Lys Thr Glu Ala Val Val Ser Ser
 50 55 60
 Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu
 65 70 75 80
 Ser Tyr Lys Glu Lys Phe Ile Val Arg Cys Tyr Glu Val Gly Ile Asn
 85 90 95
 Lys Thr Ala Thr Val Glu Thr Ile Ala Asn Leu Leu Gln Glu Val Val
 100 105 110
 Cys Asn His Ala Gln Ser Val Gly Tyr Ser Thr Gly Gly Phe Ser Thr
 115 120 125
 Thr Pro Thr Met Arg Lys Leu Arg Leu Ile Trp Val Thr Ala Arg Met
 130 135 140
 His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu Ile
 145 150 155 160
 Glu Ser Trp Gly Gln Gly Glu Gly Lys Ile Gly Thr Arg Arg Asp Trp
 165 170 175
 Ile Leu Arg Asp Tyr Ala Thr Gly Gln Val Ile Gly Arg Ala Thr Ser
 180 185 190
 Lys Trp Val Met Met Asn Gln Asp Thr Arg Arg Leu Gln Lys Val Asp
 195 200 205
 Val Asp Val Arg Asp Glu Tyr Leu Val His Cys Pro Arg Glu Leu Arg
 210 215 220
 Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys
 225 230 235 240
 Leu Glu Asp Pro Ser Gln Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg
 245 250 255
 Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile Gly
 260 265 270
 Trp Val Leu Glu Ser Met Pro Gln Glu Ile Ile Asp Thr His Glu Leu
 275 280 285
 Gln Thr Ile Thr Leu Asp Tyr Arg Arg Glu Cys Gln His Asp Asp Val
 290 295 300
 Val Asp Ser Leu Thr Ser Pro Glu Pro Ser Glu Asp Ala Glu Ala Val
 305 310 315 320
 Phe Asn His Asn Gly Thr Asn Gly Ser Ala Asn Val Ser Ala Asn Asp
 325 330 335
 His Gly Cys Arg Asn Phe Leu His Leu Leu Arg Leu Ser Gly Asn Gly
 340 345 350
 Leu Glu Ile Asn Arg Gly Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
 355 360 365
 Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
 370 375 380
 Tyr Lys Asp Asp Asp Asp Lys
 385 390

<210> SEQ ID NO 21

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<211> LENGTH: 391
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 21

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30
 Pro Val Arg Gly Arg Ala Ile Pro Pro Arg Ile Ile Val Val Ser Ser
 35 40 45
 Ser Ser Ser Lys Val Asn Pro Leu Lys Thr Glu Ala Val Val Ser Ser
 50 55 60
 Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu
 65 70 75 80
 Ser Tyr Lys Glu Lys Phe Ile Val Arg Cys Tyr Glu Val Gly Ile Asn
 85 90 95
 Lys Thr Ala Thr Val Glu Thr Ile Ala Asn Leu Leu Gln Glu Val Gly
 100 105 110
 Cys Asn His Ala Gln Ser Val Gly Tyr Ser Thr Ala Gly Phe Ser Thr
 115 120 125
 Thr Pro Thr Met Arg Lys Leu Arg Leu Ile Trp Val Thr Ala Arg Met
 130 135 140
 His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu Ile
 145 150 155 160
 Glu Ser Trp Gly Gln Gly Glu Gly Lys Ile Gly Thr Arg Arg Asp Trp
 165 170 175
 Ile Leu Arg Asp Tyr Ala Thr Gly Gln Val Ile Gly Arg Ala Thr Ser
 180 185 190
 Lys Trp Val Met Met Asn Gln Asp Thr Arg Arg Leu Gln Lys Val Asp
 195 200 205
 Val Asp Val Arg Asp Glu Tyr Leu Val His Cys Pro Arg Glu Leu Arg
 210 215 220
 Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys
 225 230 235 240
 Leu Glu Asp Pro Ser Gln Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg
 245 250 255
 Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile Gly
 260 265 270
 Trp Val Leu Glu Ser Met Pro Gln Glu Ile Ile Asp Thr His Glu Leu
 275 280 285
 Gln Thr Ile Thr Leu Asp Tyr Arg Arg Glu Cys Gln His Asp Asp Val
 290 295 300
 Val Asp Ser Leu Thr Ser Pro Glu Pro Ser Glu Asp Ala Glu Ala Val
 305 310 315 320
 Phe Asn His Asn Gly Thr Asn Gly Ser Ala Asn Val Ser Ala Asn Asp
 325 330 335
 His Gly Cys Arg Asn Phe Leu His Leu Leu Arg Leu Ser Gly Asn Gly
 340 345 350
 Leu Glu Ile Asn Arg Gly Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
 355 360 365
 Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp

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His Gly Cys Arg Asn Phe Leu His Leu Leu Arg Leu Ser Gly Asn Gly
 340 345 350

Leu Glu Ile Asn Arg Gly Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
 355 360 365

Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
 370 375 380

Tyr Lys Asp Asp Asp Asp Lys
 385 390

<210> SEQ ID NO 23
 <211> LENGTH: 391
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polypeptide

<400> SEQUENCE: 23

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15

Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30

Pro Val Arg Gly Arg Ala Ile Pro Pro Arg Ile Ile Val Val Ser Ser
 35 40 45

Ser Ser Ser Lys Val Asn Pro Leu Lys Thr Glu Ala Val Val Ser Ser
 50 55 60

Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu
 65 70 75 80

Ser Tyr Lys Glu Lys Phe Ile Val Arg Cys Tyr Glu Val Gly Ile Asn
 85 90 95

Lys Thr Ala Thr Val Glu Thr Ile Ala Asn Lys Leu Gln Glu Val Gly
 100 105 110

Cys Asn His Ala Gln Ser Val Gly Tyr Ser Thr Gly Gly Phe Ser Thr
 115 120 125

Thr Pro Thr Met Arg Lys Leu Arg Leu Ile Trp Val Thr Ala Arg Met
 130 135 140

His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu Ile
 145 150 155 160

Glu Ser Trp Gly Gln Gly Glu Gly Lys Ile Gly Thr Arg Arg Asp Trp
 165 170 175

Ile Leu Arg Asp Tyr Ala Thr Gly Gln Val Ile Gly Arg Ala Thr Ser
 180 185 190

Lys Trp Val Met Met Asn Gln Asp Thr Arg Arg Leu Gln Lys Val Asp
 195 200 205

Val Asp Val Arg Asp Glu Tyr Leu Val His Cys Pro Arg Glu Leu Arg
 210 215 220

Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys
 225 230 235 240

Leu Glu Asp Pro Ser Gln Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg
 245 250 255

Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile Gly
 260 265 270

Trp Val Leu Glu Ser Met Pro Gln Glu Ile Ile Asp Thr His Glu Leu
 275 280 285

Gln Thr Ile Thr Leu Asp Tyr Arg Arg Glu Cys Gln His Asp Asp Val
 290 295 300

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Val Asp Ser Leu Thr Ser Pro Glu Pro Ser Glu Asp Ala Glu Ala Val
 305 310 315 320
 Phe Asn His Asn Gly Thr Asn Gly Ser Ala Asn Val Ser Ala Asn Asp
 325 330 335
 His Gly Cys Arg Asn Phe Leu His Leu Leu Arg Leu Ser Gly Asn Gly
 340 345 350
 Leu Glu Ile Asn Arg Gly Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
 355 360 365
 Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
 370 375 380
 Tyr Lys Asp Asp Asp Asp Lys
 385 390

<210> SEQ ID NO 24

<211> LENGTH: 391

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 24

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30
 Pro Val Arg Gly Arg Ala Ile Pro Pro Arg Ile Ile Val Val Ser Ser
 35 40 45
 Ser Ser Ser Lys Val Asn Pro Leu Lys Thr Glu Ala Val Val Ser Ser
 50 55 60
 Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu
 65 70 75 80
 Ser Tyr Lys Glu Lys Phe Ile Val Arg Cys Tyr Glu Val Gly Ile Asn
 85 90 95
 Lys Thr Ala Thr Val Glu Thr Ile Ala Asn Ser Leu Gln Glu Val Gly
 100 105 110
 Cys Asn His Ala Gln Ser Val Gly Tyr Ser Thr Gly Gly Phe Ser Thr
 115 120 125
 Thr Pro Thr Met Arg Lys Leu Arg Leu Ile Trp Val Thr Ala Arg Met
 130 135 140
 His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu Ile
 145 150 155 160
 Glu Ser Trp Gly Gln Gly Glu Gly Lys Ile Gly Thr Arg Arg Asp Trp
 165 170 175
 Ile Leu Arg Asp Tyr Ala Thr Gly Gln Val Ile Gly Arg Ala Thr Ser
 180 185 190
 Lys Trp Val Met Met Asn Gln Asp Thr Arg Arg Leu Gln Lys Val Asp
 195 200 205
 Val Asp Val Arg Asp Glu Tyr Leu Val His Cys Pro Arg Glu Leu Arg
 210 215 220
 Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys
 225 230 235 240
 Leu Glu Asp Pro Ser Gln Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg
 245 250 255
 Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile Gly

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260					265					270					
Trp	Val	Leu	Glu	Ser	Met	Pro	Gln	Glu	Ile	Ile	Asp	Thr	His	Glu	Leu
	275						280					285			
Gln	Thr	Ile	Thr	Leu	Asp	Tyr	Arg	Arg	Glu	Cys	Gln	His	Asp	Asp	Val
	290					295					300				
Val	Asp	Ser	Leu	Thr	Ser	Pro	Glu	Pro	Ser	Glu	Asp	Ala	Glu	Ala	Val
	305				310					315					320
Phe	Asn	His	Asn	Gly	Thr	Asn	Gly	Ser	Ala	Asn	Val	Ser	Ala	Asn	Asp
				325					330					335	
His	Gly	Cys	Arg	Asn	Phe	Leu	His	Leu	Leu	Arg	Leu	Ser	Gly	Asn	Gly
			340					345					350		
Leu	Glu	Ile	Asn	Arg	Gly	Arg	Thr	Glu	Trp	Arg	Lys	Lys	Pro	Thr	Arg
		355					360						365		
Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp
	370					375						380			
Tyr	Lys	Asp	Asp	Asp	Asp	Lys									
	385				390										

<210> SEQ ID NO 25

<211> LENGTH: 391

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 25

Met	Ala	Thr	Ala	Ser	Thr	Phe	Ser	Ala	Phe	Asn	Ala	Arg	Cys	Gly	Asp
1				5					10					15	
Leu	Arg	Arg	Ser	Ala	Gly	Ser	Gly	Pro	Arg	Arg	Pro	Ala	Arg	Pro	Leu
			20					25					30		
Pro	Val	Arg	Gly	Arg	Ala	Ile	Pro	Pro	Arg	Ile	Ile	Val	Val	Ser	Ser
			35				40					45			
Ser	Ser	Ser	Lys	Val	Asn	Pro	Leu	Lys	Thr	Glu	Ala	Val	Val	Ser	Ser
			50			55				60					
Gly	Leu	Ala	Asp	Arg	Leu	Arg	Leu	Gly	Ser	Leu	Thr	Glu	Asp	Gly	Leu
					70					75					80
Ser	Tyr	Lys	Glu	Lys	Phe	Ile	Val	Arg	Cys	Tyr	Glu	Val	Gly	Ile	Asn
				85					90					95	
Lys	Thr	Ala	Thr	Val	Glu	Thr	Ile	Ala	Asn	Leu	Leu	Gln	Glu	Val	Gly
			100					105					110		
Cys	Asn	His	Ala	Gln	Ser	Val	Gly	Tyr	Ser	Thr	Val	Gly	Phe	Ser	Thr
			115				120						125		
Thr	Pro	Thr	Met	Arg	Lys	Leu	Arg	Leu	Ile	Trp	Val	Thr	Ala	Arg	Met
			130			135					140				
His	Ile	Glu	Ile	Tyr	Lys	Tyr	Pro	Ala	Trp	Ser	Asp	Val	Val	Glu	Ile
					150					155					160
Glu	Ser	Trp	Gly	Gln	Gly	Glu	Gly	Lys	Ile	Gly	Thr	Arg	Arg	Asp	Trp
				165					170						175
Ile	Leu	Arg	Asp	Tyr	Ala	Thr	Gly	Gln	Val	Ile	Gly	Arg	Ala	Thr	Ser
			180					185						190	
Lys	Trp	Val	Met	Met	Asn	Gln	Asp	Thr	Arg	Arg	Leu	Gln	Lys	Val	Asp
		195					200						205		
Val	Asp	Val	Arg	Asp	Glu	Tyr	Leu	Val	His	Cys	Pro	Arg	Glu	Leu	Arg
					210		215					220			

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Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys
 225 230 235 240
 Leu Glu Asp Pro Ser Gln Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg
 245 250 255
 Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile Gly
 260 265 270
 Trp Val Leu Glu Ser Met Pro Gln Glu Ile Ile Asp Thr His Glu Leu
 275 280 285
 Gln Thr Ile Thr Leu Asp Tyr Arg Arg Glu Cys Gln His Asp Asp Val
 290 295 300
 Val Asp Ser Leu Thr Ser Pro Glu Pro Ser Glu Asp Ala Glu Ala Val
 305 310 315 320
 Phe Asn His Asn Gly Thr Asn Gly Ser Ala Asn Val Ser Ala Asn Asp
 325 330 335
 His Gly Cys Arg Asn Phe Leu His Leu Leu Arg Leu Ser Gly Asn Gly
 340 345 350
 Leu Glu Ile Asn Arg Gly Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
 355 360 365
 Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
 370 375 380
 Tyr Lys Asp Asp Asp Lys
 385 390

<210> SEQ ID NO 26
 <211> LENGTH: 391
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polypeptide

<400> SEQUENCE: 26

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30
 Pro Val Arg Gly Arg Ala Ile Pro Pro Arg Ile Ile Val Val Ser Ser
 35 40 45
 Ser Ser Ser Lys Val Asn Pro Leu Lys Thr Glu Ala Val Val Ser Ser
 50 55 60
 Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu
 65 70 75 80
 Ser Tyr Lys Glu Lys Phe Ile Val Arg Cys Tyr Glu Val Gly Ile Asn
 85 90 95
 Lys Thr Ala Thr Val Glu Thr Ile Ala Asn Leu Leu Gln Glu Val Gly
 100 105 110
 Cys Asn His Ala Gln Ser Val Gly Tyr Ser Thr Gly Gly Phe Ser Thr
 115 120 125
 Thr Pro Thr Met Arg Lys Leu Arg Leu Ile Trp Val Thr Ala Arg Met
 130 135 140
 His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu Ile
 145 150 155 160
 Glu Ser Trp Gly Gln Gly Glu Gly Lys Ile Gly Phe Arg Arg Asp Trp
 165 170 175
 Ile Leu Arg Asp Tyr Ala Thr Gly Gln Val Ile Gly Arg Ala Thr Ser
 180 185 190

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Lys Trp Val Met Met Asn Gln Asp Thr Arg Arg Leu Gln Lys Val Asp
 195 200 205
 Val Asp Val Arg Asp Glu Tyr Leu Val His Cys Pro Arg Glu Leu Arg
 210 215 220
 Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys
 225 230 235 240
 Leu Glu Asp Pro Ser Gln Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg
 245 250 255
 Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile Gly
 260 265 270
 Trp Val Leu Glu Ser Met Pro Gln Glu Ile Ile Asp Thr His Glu Leu
 275 280 285
 Gln Thr Ile Thr Leu Asp Tyr Arg Arg Glu Cys Gln His Asp Asp Val
 290 295 300
 Val Asp Ser Leu Thr Ser Pro Glu Pro Ser Glu Asp Ala Glu Ala Val
 305 310 315 320
 Phe Asn His Asn Gly Thr Asn Gly Ser Ala Asn Val Ser Ala Asn Asp
 325 330 335
 His Gly Cys Arg Asn Phe Leu His Leu Leu Arg Leu Ser Gly Asn Gly
 340 345 350
 Leu Glu Ile Asn Arg Gly Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
 355 360 365
 Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
 370 375 380
 Tyr Lys Asp Asp Asp Asp Lys
 385 390

<210> SEQ ID NO 27
 <211> LENGTH: 391
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polypeptide

<400> SEQUENCE: 27

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30
 Pro Val Arg Gly Arg Ala Ile Pro Pro Arg Ile Ile Val Val Ser Ser
 35 40 45
 Ser Ser Ser Lys Val Asn Pro Leu Lys Thr Glu Ala Val Val Ser Ser
 50 55 60
 Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu
 65 70 75 80
 Ser Tyr Lys Glu Lys Phe Ile Val Arg Cys Tyr Glu Val Gly Ile Asn
 85 90 95
 Lys Thr Ala Thr Val Glu Thr Ile Ala Asn Leu Leu Gln Glu Val Gly
 100 105 110
 Cys Asn His Ala Gln Ser Val Gly Tyr Ser Thr Gly Gly Phe Ser Thr
 115 120 125
 Thr Pro Thr Met Arg Lys Leu Arg Leu Ile Trp Val Thr Ala Arg Met
 130 135 140
 His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu Ile

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Cys Asn His Ala Gln Ser Val Gly Tyr Ser Thr Gly Gly Phe Ser Thr
 115 120 125
 Thr Pro Thr Met Arg Lys Leu Arg Leu Ile Trp Val Thr Ala Arg Met
 130 135 140
 His Ile Glu Ile Tyr Lys Tyr Pro Ala Trp Ser Asp Val Val Glu Ile
 145 150 155 160
 Glu Ser Trp Gly Gln Gly Glu Gly Lys Ile Gly Lys Arg Arg Asp Trp
 165 170 175
 Ile Leu Arg Asp Tyr Ala Thr Gly Gln Val Ile Gly Arg Ala Thr Ser
 180 185 190
 Lys Trp Val Met Met Asn Gln Asp Thr Arg Arg Leu Gln Lys Val Asp
 195 200 205
 Val Asp Val Arg Asp Glu Tyr Leu Val His Cys Pro Arg Glu Leu Arg
 210 215 220
 Leu Ala Phe Pro Glu Glu Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys
 225 230 235 240
 Leu Glu Asp Pro Ser Gln Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg
 245 250 255
 Ala Asp Leu Asp Met Asn Gln His Val Asn Asn Val Thr Tyr Ile Gly
 260 265 270
 Trp Val Leu Glu Ser Met Pro Gln Glu Ile Ile Asp Thr His Glu Leu
 275 280 285
 Gln Thr Ile Thr Leu Asp Tyr Arg Arg Glu Cys Gln His Asp Asp Val
 290 295 300
 Val Asp Ser Leu Thr Ser Pro Glu Pro Ser Glu Asp Ala Glu Ala Val
 305 310 315 320
 Phe Asn His Asn Gly Thr Asn Gly Ser Ala Asn Val Ser Ala Asn Asp
 325 330 335
 His Gly Cys Arg Asn Phe Leu His Leu Leu Arg Leu Ser Gly Asn Gly
 340 345 350
 Leu Glu Ile Asn Arg Gly Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
 355 360 365
 Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
 370 375 380
 Tyr Lys Asp Asp Asp Asp Lys
 385 390

<210> SEQ ID NO 29

<211> LENGTH: 391

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 29

Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
 1 5 10 15
 Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
 20 25 30
 Pro Val Arg Gly Arg Ala Ile Pro Pro Arg Ile Ile Val Val Ser Ser
 35 40 45
 Ser Ser Ser Lys Val Asn Pro Leu Lys Thr Glu Ala Val Val Ser Ser
 50 55 60
 Gly Leu Ala Asp Arg Leu Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu
 65 70 75 80

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ttttcttaaa gcaaatgact gctgattgac cagatactgt aacgctgatt tegctccaga	300
tcgcacagat agcgaccatg ttgctgcgtc tgaaaaactg gattccgaat tcgaccctgg	360
cgctccatcc atgcaacaga tggcgacact tgttacaatt cctgtcacc ctcggcatgg	420
agcagggtcca cttagattcc cgatcaccca cgcacatctc gctaatagtc attcgttcgt	480
gtcttcgatc aatctcaagt gactgtgcat ggatcttggg tgacgatgcg gtatggggtt	540
gcgccgctgg ctgcagggtc tgcccgaagg aagctaacc agctcctctc cccgacaata	600
ctctcgcagg caaagccggg cacttgctt ccagattgcc aataaactca attatggcct	660
ctgtcatgcc atccatgggt ctgatgaatg gtcacgctcg tgtcctgacc gttcccagc	720
ctctggcgtc cctgcctccg cccaccagcc cagccgcgc ggcagtcgct gccaaaggctg	780
tctcggagggt accctttctt gcgctatgac acttccagca aaaggtaggg cgggctgca	840
gacggcttcc cggcgctgca tgcaacaccg atgatgcttc gacccccga agctccttcg	900
gggctgcatg ggcgctccga tgcgctcca gggcgagcgc tgtttaaata gccaggcccc	960
cgattgcaaa gacattatag cgagctacca aagccatatt caaacaccta gatcactacc	1020
acttctacac aggccactcg agcttgtgat cgcactccgc taagggggcg cctcttctc	1080
ttcgtttcag tcacaaccg caaactctag aatatcaatg atcgagcagg acggcctcca	1140
cgcggctcc cccgcgctt ggggtgagcg cctgttcggc tacgactggg cccagcagac	1200
catcggctgc tccgacgccc ccgtgttccg cctgtccgcc caggccgccc ccgtgctgtt	1260
cgtgaagacc gacctgtccg gcgcccgaag cgagctgcag gacgaggccc cccgcctgtc	1320
ctggctggcc accaccggcg tgcctgcgc cgcctgctg gagctgggta ccgaggccgg	1380
ccgcgactgg ctgctgctgg gcgaggtgcc cggccaggac ctgctgtcct cccacctggc	1440
ccccgcgag aagggtgtcca tcattggcca gcacatgcgc cgcctgcaca cctggacc	1500
cgccacctgc cctctgacc accaggccaa gcaccgcatc gagcgcgccc gcaccgcat	1560
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cgagctgttc gccgcctga agcccccgc gcccagcgc gaggacctgg tggtgaccca	1680
cggcgacgcc tgcttgcaca acatcatggt ggagaacggc cgttctccg gcttcatcga	1740
ctgcggccc ctgggctgg ccgaccgcta ccaggacatc gccctggcca cccgcgacat	1800
cgccgaggag ctggggggcg agtgggcca cgccttcctg gtgctgtacg gcatcgcgc	1860
ccccgactcc cagcgcacg ccttctacc cctgctggac gagttctct gacaattgac	1920
gccccgcgg cgcacctgac ctgttctctc gagggcgctt gttctgcctt gcgaaacaag	1980
ccccgggag atcgctgcat gatcgtctct ggcgccccgc cgcgcggttt gtcgcccctg	2040
cgggcgccc ggcgcgggg gcgcattgaa attgttgcaa accccacctg acagattgag	2100
ggcccaggca ggaaggcgtt gagatggagg tacaggagtc aagtaactga aagttttat	2160
gataactaac aacaaagggt cgtttctggc cagcgaatga caagaacaag attccacatt	2220
tccgtgtaga ggcttgcac cgaatgtgag cgggggggccc gcggaccga caaaaccctt	2280
acgacgtggt aagaaaaacg tggcgggac tgctcctgta gcctgaagac cagcaggaga	2340
cgatcggaa catcacagca caggatcccg cgtctcgaac agagcgcgca gaggaacgct	2400
gaaggtctcg cctctgtcgc acctcagcgc ggcatacacc acaataacca cctgacgaat	2460
gcgcttggtt ctctgctcat tagcgaagcg tccggttcac acacgtgcca cgttggcgag	2520
gtggcaggty acaatgatcg gtggagctga tggtcgaaac gttcacagcc tagggatatc	2580

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gtgaaaactc gctcgcaccg cgcggtcccc caggcagcga tgacgtgtgc gtgacctggg	2640
tgtttcgtcg aaaggccagc aacccccaaat cgcaggcgat cgggagattg ggatctgatc	2700
cgagcttgga ccagatcccc cagcatgcgg cacgggaact gcatcgactc ggcgcggaac	2760
ccagctttcg taaatgccag attggtgtcc gatacctga tttgccatca gcgaaacaag	2820
acttcagcag cgagcgtatt tggcggggct gctaccaggg ttgcatacat tgcccatttc	2880
tgtctggacc gctttaccgg cgcagagggt gagttgatgg gggtggcagg catcgaaacg	2940
cgcgtgcatg gtgtgtgtgt ctgttttcgg ctgcacaatt tcaatagtcg gatgggcgac	3000
ggtagaattg ggtgttcgct tcgctgcat gcctcgcccc gtcgggtgtc atgacctgga	3060
ctggaatccc ccctcgcgac cctcctgcta acgctccga ctctcccgc cgcgcgagc	3120
atagactcta gttcaaccaa tcgacaacta gtatggccac cgcattccact ttctcggcgt	3180
tcaatgcccc ctgcggcgac ctgcgtcgtc cggcgggctc cgggccccgg cgcccagcga	3240
ggccccctcc cgtgcgcggg cgcgccatcc cccccgcat catcgtgggtg tctctctct	3300
cctccaaggt gaacccccctg aagaccgagg ccgtgggtgtc ctccggcctg gccgaccgcc	3360
tgcgcctggg ctccctgacc gaggacggcc tgtcctacaa ggagaagttc atcgtgcgct	3420
gctacgaggt gggcatcaac aagaccgcca ccgtggagac catcgccaac ctgctgcagg	3480
aggtgggctg caaccacgcc cagtccgtgg gctactccac cggcggcttc tccaccacc	3540
ccaccatgcg caagctgcgc ctgatctggg tgaccgccc catgcacatc gagatctaca	3600
agtacccccg ctggtccgac gtggtggaga tcgagtcctg gggccagggc gagggcaaga	3660
tcggcaccog ccgcgactgg atcctgcgcg actacgccac cggccagggtg atcggccgcg	3720
ccacctcaa gtgggtgatg atgaaccagg acaccgccc cctgcagaag gtggacgtgg	3780
acgtgcgca cgagtacctg gtgcaactgc cccgcgagct gcgcctggcc ttcccagag	3840
agaacaactc ctccctgaag aagatctcca agctggagga cccctcccag tactccaagc	3900
tgggcctggg gccccgcgc gccgacctgg acatgaacca gcacgtgaac aacgtgacct	3960
acatcggtcg ggtgctggag tccatgcccc aggagatcat cgacaccac gagctgcaga	4020
ccatcacctt ggactaccgc cgcgagtgcc agcacgacga cgtggtggac tccctgacct	4080
cccccgagcc ctccgaggac gccgagggcg tgttcaacca caacggcacc aacggctccg	4140
ccaacgtgtc cgccaacgac cacggtgccc gcaacttctt gcaactgctg cgcctgtccg	4200
gcaacggcct ggagatcaac cgcggccgca ccgagtggcg caagaagccc acccgcatgg	4260
actacaagga ccacgacggc gactacaagg accacgacat cgactacaag gacgacgacg	4320
acaagtgaat cgatgcagca gcagctcgga tagtatcgac aactctgga cgtggtcgt	4380
gtgatggact gttgccgcca cacttctgtc cttgacctgt gaatatccct gccgctttta	4440
tcaaacagcc tcagtgtgtt tgatcttggt tgtaacgctt tttgcgagtt gctagctgct	4500
tgtgtatatt gcgaatacca cccccagcat ccccttccct cgtttcatat cgtttgcatc	4560
ccaaccgcaa cttatctacg ctgtctgtct atccctcagc gctgctcctg ctctgtctca	4620
ctgcccctcg cacagccttg gtttgggctc cgcctgtatt ctccctgtac tgcaacctgt	4680
aaaccagcac tgcaatgctg atgcacggga agtagtggga tgggaacaca aatggaaagc	4740
ttgagctcca gcgccatgcc acgccccttg atggettcaa gtaacgattac ggtggtggat	4800
tgtgtgtttg ttgcgtagtg tgcattggtt agaataatac acttgatttc ttgctcacgg	4860
caatctcggc ttgtccgag gttcaacccc atttcggagt ctcaggtcag ccgccaatg	4920
accagccgct acttcaagga cttgcacgac aacgcgagg tgagctatgt ttaggacttg	4980

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attggaatt gtcgtcgacg catattcgcg ctccgcgaca gcaccaagc aaaatgtcaa 5040
gtgcttccg atttgcgtcc gcaggtcgat gttgtgatcg tcggcgccgg atccgcccgt 5100
ctgtcctcgc cttacgagct gaccaagcac cctgacgtcc gggtagcgca gctgagattc 5160
gattagacat aaattgaaga ttaaaccctg agaaaaattt gatggtcgcg aaactgtgct 5220
cgattgcaag aaattgatcg tcctccactc cgcaggtcgc catcatcgag cagggcgctg 5280
ctcccggcgg cggcgccctg ctggggggac agctgttctc ggccatgtgt gtacgtagaa 5340
ggatgaattt cagctggttt tcgttgacaca gctgtttgtg catgatttgt ttcagactat 5400
tgttgaatgt ttttagattt cttaggatgc atgatttgtc tgcatgcgac t 5451

```

```

<210> SEQ ID NO 31
<211> LENGTH: 1176
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

```

```

<400> SEQUENCE: 31

```

```

atggccaccg caccacttt ctggcgcttc aatgcccgtc gggcgacct gcgtcgctcg 60
gggggctcgg gggcccgggc cccagcaggg cccctccccg tgcgcgggcg cgccatcccc 120
ccccgcatca tcgtggtgtc ctctctctcc tccaaggta acccctgaa gaccgaggcc 180
gtggtgtcct cggcctggc cgaccgctc gcctgggct ccctgaccga ggacggcctg 240
tcctacaagg agaagtcat cgtgcgctgc tacgaggtgg gcatcaacaa gaccgccacc 300
gtggagacca tcgccaacct gctgcaggag gtgggctgca accacgcccc gtccgtgggc 360
tactccaccg gggccttcgc caccaccccc accatgcgca agctgcgctc gatctgggtg 420
accgcccgca tgcacatcga gatctacaag taccgccctc ggtccgacgt ggtggagatc 480
gagtcctggg gccaggcgca gggcaagatc ggcacccgcc gcgactggat cctgcgcgac 540
tacgccaccg gccaggtgat cggccgcgcc acctccaagt gggatgatga gaaccaggac 600
accgcccgcc tgcagaaggt ggacgcggac gtgcgcgacg agtacctggt gcaactgccc 660
cgcgactgca gcctggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag 720
ctggaggacc cctcccagta ctccaagctg ggcctgggtc cccgcccgcg cgacctggac 780
atgaaccagc acgtgaacaa cgtgacctac atcggctggg tgctggagtc catgccccag 840
gagatcatcg acaccacgca gctgcagacc atcaccctgg actaccgccc cgagtgccag 900
cacgacgacg tgggtgactc cctgacctcc cccgagccct ccgaggacgc cgaggccgtg 960
ttcaaccaca acggcaccac cggtctccgc aacgtgtccg ccaacgacca cggtgcccgc 1020
aacttctcgc acctgctcgc cctgtccggc aacggcctgg agatcaaccg cggccgcacc 1080
gagtgccgca agaagcccac ccgcatggac tacaaggacc acgacggcga ctacaaggac 1140
cacgacatcg actacaagga cgacgacgac aagtga 1176

```

```

<210> SEQ ID NO 32
<211> LENGTH: 1176
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

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<400> SEQUENCE: 32

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atggccacg catccacttt ctggcggttc aatgcccgcg gcggcgacct gcgtcgctcg    60
gctggctccg gggcccggcg cccagcgagg cccctcccgc tgcgctggcg cgccatcccc    120
ccccgcatca tcgtggtgtc ctctctctcc tccaagggtga accccctgaa gaccgaggcc    180
gtggtgtcct cggcctggc cgaccgctg cgctgggct ccctgaccga ggaaggctg    240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaacaa gaccgccacc    300
gtggagacca tcgccaacct gctgcaggag gtggctgca accacgccc gtcctggggc    360
tactccacg gcgcttctg caccacccc accatgcgca agctgcgct gatctgggtg    420
accgcccgca tgcacatoga gatctacaag taccgcccgt ggtccgacgt ggtggagatc    480
gagtcctggg gccagggcga gggcaagatc ggcaccccgc gcgactggat cctgcgagac    540
tacgccacg gccaggtgat cggcccgccc acctccaagt gggatgatgat gaaccaggac    600
accgcccgca tgcagaaggt ggacgaggac gtgcgagac agtacctggt gactgcccc    660
cgcgagctgc gctggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag    720
ctggaggacc cctcccagta ctccaagctg ggcctggtgc cccgcccgcg cgacctggac    780
atgaaccagc acgtgaacaa cgtgacctac atcggctggg tgctggagtc catgcccag    840
gagatcatcg acaccacga gctgcagacc atcaccctgg actaccgccc cgagtgccag    900
cacgacgacg tgggtgactc cctgacctcc cccgagcct ccgaggacgc cgaggccgtg    960
ttcaaccaca acggcaccaa cggctccgccc aacgtgtccc ccaacgacca cggctgccc    1020
aacttctgca acctgctgca cctgtccgca aacggcctgg agatcaaccg cggccgccc    1080
gagtgccgca agaagcccac ccgcatggac tacaaggacc acgacggcga ctacaaggac    1140
cacgacatcg actacaagga cgacgacgac aagtga                                1176

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<210> SEQ ID NO 33

<211> LENGTH: 1176

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 33

```

atggccacg catccacttt ctggcggttc aatgcccgcg gcggcgacct gcgtcgctcg    60
gctggctccg gggcccggcg cccagcgagg cccctcccgc tgcgctggcg cgccatcccc    120
ccccgcatca tcgtggtgtc ctctctctcc tccaagggtga accccctgaa gaccgaggcc    180
gtggtgtcct cggcctggc cgaccgctg cgctgggct ccctgaccga ggaaggctg    240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaacaa gaccgccacc    300
gtggagacca tcgccaacct gctgcaggag gtggctgca accacgccc gtcctggggc    360
tactccacg gcgcttctc caccacccc accatgcgca agctgcgct gatctgggtg    420
accgcccgca tgcacatoga gatctacaag taccgcccgt ggtccgacgt ggtggagatc    480
gagtcctggg gccagggcga gggcaagatc ggcaccccgc gcgactggat cctgcgagac    540
tacgccacg gccaggtgat cggcccgccc acctccaagt gggatgatgat gaaccaggac    600
accgcccgca tgcagaaggt ggacgtggac gtgcgagac agtacctggt gactgcccc    660
cgcgagctgc gctggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag    720
ctggaggacc cctcccagta ctccaagctg ggcctggtgc cccgcccgcg cgacctggac    780
atgaaccagc acgtgaacaa cgtgacctac atcggctggg tgctggagtc catgcccag    840

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gagatcatcg acaccacga gctgcagacc atcacctgg actaccgcc cgagtgccag 900
cacgacgacg tggtggaetc cctgacctcc cccgagccct ccgaggacgc cgaggccgtg 960
ttcaaccaca acggcaccaa cggctccgcc aacgtgtccg ccaacgacca cggctgccgc 1020
aacttcctgc acctgctgcy cctgtccggc aacggcctgg agatcaaccy cggccgcacc 1080
gagtggcgca agaagcccac ccgcatggac tacaaggacc acgacggcga ctacaaggac 1140
cacgacatcg actacaagga cgacgacgac aagtga 1176

```

```

<210> SEQ ID NO 34
<211> LENGTH: 1176
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
                        polynucleotide

```

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<400> SEQUENCE: 34

```

```

atggccaccg catcacttt ctggcggttc aatgcccgt gcggcgacct gcgtcgctcg 60
gcgggctccg ggccccggcg cccagcgagg cccctcccc tgcgcgggcg cgccatcccc 120
ccccgcatca tcgtggtgtc ctctctctcc tccaaggatg acccctgaa gaccgaggcc 180
gtggtgtcct ccggcctggc cgaccgcctg cgcctgggct ccctgaccga ggacggcctg 240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaaaaa gaccgccacc 300
gtggagacca tcgccaacct gctgcaggag gtgacgtgca accacgccc gtcctggggc 360
tactccaccg gcgcttctc caccaccccc accatgcgca agctgcgctc gatctgggtg 420
accgcccgca tgcacatoga gatctacaag taccocgctt ggtccgacgt ggtggagatc 480
gagtcctggg gccagggcga gggcaagatc ggcacccgcc gcgactggat cctgcgcgac 540
tacgccaccg gccaggtgat cggccgcgcc acctccaagt gggatgatgat gaaccaggac 600
accgcccgcc tgcagaaggt ggacgtggac gtgcgcgacg agtacctggt gcactgcccc 660
cgcgagctgc gcctggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag 720
ctggaggacc cctcccagta ctccaagctg ggcctgggtc cccgcccgcg cgacctggac 780
atgaaccagc acgtgaacaa cgtgacctac atcggtggg tgctggagtc catgcccag 840
gagatcatcg acaccacga gctgcagacc atcacctgg actaccgcc cgagtgccag 900
cacgacgacg tggtggaetc cctgacctcc cccgagccct ccgaggacgc cgaggccgtg 960
ttcaaccaca acggcaccaa cggctccgcc aacgtgtccg ccaacgacca cggctgccgc 1020
aacttcctgc acctgctgcy cctgtccggc aacggcctgg agatcaaccy cggccgcacc 1080
gagtggcgca agaagcccac ccgcatggac tacaaggacc acgacggcga ctacaaggac 1140
cacgacatcg actacaagga cgacgacgac aagtga 1176

```

```

<210> SEQ ID NO 35
<211> LENGTH: 1176
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
                        polynucleotide

```

```

<400> SEQUENCE: 35

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```

atggccaccg catecacttt ctggcggttc aatgcccgt gcggcgacct gcgtcgctcg 60
gcgggctccg ggccccggcg cccagcgagg cccctcccc tgcgcgggcg cgccatcccc 120
ccccgcatca tcgtggtgtc ctctctctcc tccaaggatg acccctgaa gaccgaggcc 180

```

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```

gtggtgtcct cggcctggc cgaccgctg cgcctgggt ccctgaccga ggacggcctg 240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaacaa gaccgccacc 300
gtggagacca tcgccaacct gctgcaggag gtggtgtgca accacgcccc gtccgtgggc 360
tactccaccg cggccttctc caccaccccc accatgcgca agctgcgct gatctgggtg 420
accgcccgca tgcacatcga gatctacaag taccocgct ggtccgacgt ggtggagatc 480
gagtcctggg gccagggcga gggcaagatc ggcacccgcc gcgactggat cctgcgcgac 540
tacgccaccg gccaggtgat cggccgcgcc acctccaagt gggatgatgat gaaccaggac 600
accgcccgcc tgcagaaggt ggacgtggac gtgcgcgacg agtaacctgg gcaactgccc 660
cgcgagctgc gcctggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag 720
ctggaggacc cctcccagta ctccaagctg ggcctggtgc cccgcccgcg cgacctggac 780
atgaaccagc acgtgaacaa cgtgacctac atcggtggg tgctggagtc catgccccag 840
gagatcatcg acaccacga gctgcagacc atcacctgg actaccgccg cgagtgccag 900
cacgacgacg tggaggactc cctgacctcc cccgagcct cggaggacgc cgaggccgtg 960
ttcaaccaca acggcaccaa cggctccgcc aacgtgtccg ccaacgacca cggtgcccgc 1020
aacttcctgc acctgctgcg cctgtccggc aacggcctgg agatcaaccg cggcccacc 1080
gagtgggcga agaagccac cgcgatggac tacaaggacc acgacggcga ctacaaggac 1140
cacgacatcg actacaagga cgacgacgac aagtga 1176

```

<210> SEQ ID NO 36

<211> LENGTH: 1176

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 36

```

atggccaccg catccacttt ctggcgctc aatgcccgt gggcgacct gcgtgctcg 60
gcggtctccg gggcccggcg cccagcgagg cccctccccg tgcgggggcg cgccatcccc 120
cccccatca tcgtggtgtc ctctctctcc tccaaggta acccctgaa gaccgaggcc 180
gtggtgtcct cggcctggc cgaccgctg cgcctgggt ccctgaccga ggacggcctg 240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaacaa gaccgccacc 300
gtggagacca tcgccaacct gctgcaggag gtgggtgtgca accacgcccc gtccgtgggc 360
tactccaccg cggccttctc caccaccccc accatgcgca agctgcgct gatctgggtg 420
accgcccgca tgcacatcga gatctacaag taccocgct ggtccgacgt ggtggagatc 480
gagtcctggg gccagggcga gggcaagatc ggcacccgcc gcgactggat cctgcgcgac 540
tacgccaccg gccaggtgat cggccgcgcc acctccaagt gggatgatgat gaaccaggac 600
accgcccgcc tgcagaaggt ggacgtggac gtgcgcgacg agtaacctgg gcaactgccc 660
cgcgagctgc gcctggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag 720
ctggaggacc cctcccagta ctccaagctg ggcctggtgc cccgcccgcg cgacctggac 780
atgaaccagc acgtgaacaa cgtgacctac atcggtggg tgctggagtc catgccccag 840
gagatcatcg acaccacga gctgcagacc atcacctgg actaccgccg cgagtgccag 900
cacgacgacg tggaggactc cctgacctcc cccgagcct cggaggacgc cgaggccgtg 960
ttcaaccaca acggcaccaa cggctccgcc aacgtgtccg ccaacgacca cggtgcccgc 1020

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```

aacttctcgc acctgctgcg cctgtccggc aacggcctgg agatcaaccg cggccgcacc 1080
gagtggcgca agaagcccac ccgcatggac tacaaggacc acgacggcga ctacaaggac 1140
cacgacatcg actacaagga cgacgacgac aagtga 1176

```

```

<210> SEQ ID NO 37
<211> LENGTH: 1176
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

```

```

<400> SEQUENCE: 37

```

```

atggccacg catccacttt ctggcgcttc aatgcccgtt gcggcgacct gcgtcgctcg 60
gctggctcgg gggcccggcg cccagcggag cccctccccg tgcgctggcg cgccatcccc 120
ccccgcatca tcgtggtgtc ctctctctcc tccaaggtga acccctgaa gaccgaggcc 180
gtggtgtcct ccggcctggc cgaccgctg cgcctgggct ccctgaccga ggacggcctg 240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaacaa gaccgccacc 300
gtggagacca tcgccaactt cctgcaggag gtgggctgca accacgcccc gtccgtggcg 360
tactccacg gcggttctc caccaccccc accatgcgca agctgcgctt gatctgggtg 420
accgcccgca tgcacatcga gatctacaag taccctccct ggtccgacgt ggtggagatc 480
gagtctctgg gccagggcga gggcaagatc ggcacccgcc gcgactggat cctgcgcgac 540
tacgccacg gccaggtgat cggcccgccc acctccaagt gggatgatgat gaaccaggac 600
accgcccgcc tgcagaaggt ggacgtggac gtgcgcgacg agtacctggt gactgcccc 660
cgcgagctgc gcttggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag 720
ctggaggacc cctcccagta ctccaagctg ggcctggtgc cccgcccgcg cgacctggac 780
atgaaccagc acgtgaacaa cgtgacctac atcggtggg tgctggagtc catgccccag 840
gagatcatcg acaccacga gctgcagacc atcacctggt actaccgccc cgagtgcag 900
cacgacgacg tgggtgactc cctgacctcc cccgagccct ccgaggacgc cgaggccgtg 960
ttcaaccaca acggcaccac cggtctccgc aacgtgtccg ccaacgacca cggctgccc 1020
aacttctcgc acctgctgcg cctgtccggc aacggcctgg agatcaaccg cggccgcacc 1080
gagtggcgca agaagcccac ccgcatggac tacaaggacc acgacggcga ctacaaggac 1140
cacgacatcg actacaagga cgacgacgac aagtga 1176

```

```

<210> SEQ ID NO 38
<211> LENGTH: 1176
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

```

```

<400> SEQUENCE: 38

```

```

atggccacg catccacttt ctggcgcttc aatgcccgtt gcggcgacct gcgtcgctcg 60
gctggctcgg gggcccggcg cccagcggag cccctccccg tgcgctggcg cgccatcccc 120
ccccgcatca tcgtggtgtc ctctctctcc tccaaggtga acccctgaa gaccgaggcc 180
gtggtgtcct ccggcctggc cgaccgctg cgcctgggct ccctgaccga ggacggcctg 240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaacaa gaccgccacc 300

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gtggagacca tgcccaacaa gctgcaggag gtgggctgca accacgcca gtcogtggg	360
tactccaccg gcggttctc caccacccc accatgcgca agctgcgect gatctgggtg	420
accgcccgca tgcacatoga gatctacaag taccocgct ggtccgacgt ggtggagatc	480
gagtcctggg gccagggcga gggcaagatc ggcacccgcc gcgactggat cctgcgcgac	540
tacgccaccg gccaggtgat cggccgcgcc acctccaagt gggatgatgat gaaccaggac	600
accgcccgcc tgcagaaggt ggacgtggac gtgcgcgacg agtacctggt gcaactcccc	660
cgcgagctgc gcctggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag	720
ctggaggacc cctcccagta ctccaagctg ggcctggtgc cccgcccgcg cgacctggac	780
atgaaccagc acgtgaacaa cgtgacctac atcggtggg tgctggagtc catgcccag	840
gagatcatcg acaccacga gctgcagacc atcacctgg actaccgcc cgagtgccag	900
cacgacgacg tggaggactc cctgacctc cccgagcct ccgaggacgc cgaggccgtg	960
ttcaaccaca acggcaccaa cggctccgcc aacgtgtccg ccaacgacca cggctgccg	1020
aacttcctgc acctgctgcg cctgtccggc aacggcctgg agatcaaccg cggccgcacc	1080
gagtgccgca agaagcccac ccgcatggac tacaaggacc acgacggcga ctacaaggac	1140
cacgacatcg actacaagga cgacgacgac aagtga	1176

<210> SEQ ID NO 39

<211> LENGTH: 1176

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 39

atggccaccg catccacttt ctggcgcttc aatgcccgt ggcgacccct gcgtcgctcg	60
gcggtgctcg ggcggcgcg cccagcgagg cccctcccc tgcgcgggcg cgccatcccc	120
ccccgcatca tcgtggtgtc ctctcctcc tccaaggatga acccctgaa gaccgaggcc	180
gtggtgtcct ccggcctggc cgaccgcctg cgcctgggct ccctgaccga ggacggcctg	240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaacaa gaccgccacc	300
gtggagacca tgcccaactc gctgcaggag gtgggctgca accacgcca gtcogtggg	360
tactccaccg gcggttctc caccacccc accatgcgca agctgcgect gatctgggtg	420
accgcccgca tgcacatoga gatctacaag taccocgct ggtccgacgt ggtggagatc	480
gagtcctggg gccagggcga gggcaagatc ggcacccgcc gcgactggat cctgcgcgac	540
tacgccaccg gccaggtgat cggccgcgcc acctccaagt gggatgatgat gaaccaggac	600
accgcccgcc tgcagaaggt ggacgtggac gtgcgcgacg agtacctggt gcaactcccc	660
cgcgagctgc gcctggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag	720
ctggaggacc cctcccagta ctccaagctg ggcctggtgc cccgcccgcg cgacctggac	780
atgaaccagc acgtgaacaa cgtgacctac atcggtggg tgctggagtc catgcccag	840
gagatcatcg acaccacga gctgcagacc atcacctgg actaccgcc cgagtgccag	900
cacgacgacg tggaggactc cctgacctc cccgagcct ccgaggacgc cgaggccgtg	960
ttcaaccaca acggcaccaa cggctccgcc aacgtgtccg ccaacgacca cggctgccg	1020
aacttcctgc acctgctgcg cctgtccggc aacggcctgg agatcaaccg cggccgcacc	1080
gagtgccgca agaagcccac ccgcatggac tacaaggacc acgacggcga ctacaaggac	1140

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 cacgacatcg actacaagga cgacgacgac aagtga 1176

<210> SEQ ID NO 40
 <211> LENGTH: 1176
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polynucleotide

<400> SEQUENCE: 40

```

atggccaccg catccacttt ctggcggttc aatgcccgtc gggcgacct gcgtcgctcg      60
gggggctcgg ggccccggcg cccagcgagg cccctccccg tgcgeggggcg cgccatcccc      120
ccccgcatca tcgtggtgtc ctctctctcc tccaaggtga acccctgaa gaccgaggcc      180
gtggtgtcct ccggcctggc cgaccgctg cgcctgggct ccctgaccga ggacggcctg      240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaacaa gaccgccacc      300
gtggagacca tcgccaacct gctgcaggag gtgggctgca accacgcccc gtcctgtggc      360
tactccaccg tggccttctc caccaccccc accatgcgca agctgcgctc gatctgggtg      420
accgcccgca tgcacatcga gatctacaag taccgccctc ggtccgacgt ggtggagatc      480
gagtcctggg gccaggcgca gggcaagatc ggcacccgcc gcgactggat cctgcgcgac      540
tacgccaccg gccagggtgat cggcccgccc acctccaagt ggggtgatgat gaaccaggac      600
accgcccgcc tgcagaaggt ggacgtggac gtgcgcgacg agtacctggt gcaactgccc      660
cgcgagctgc gcctggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag      720
ctggaggacc cctcccagta ctccaagctg ggcctgggtc cccgcccgcg cgacctggac      780
atgaaccagc acgtgaacaa cgtgacctac atcggtggg tgctggagtc catgccccag      840
gagatcatcg acaccacga gctgcagacc atcaccctgg actaccgccc cgagtgccag      900
cacgacgaag tgggtgactc cctgacctcc cccgagcctc ccgaggacgc cgaggccgtg      960
ttcaaccaca acggcaccaa cggctccgcc aacgtgtccg ccaacgacca cgggtgccgc      1020
aacttctcgc acctgctgcg cctgtccggc aacggcctgg agatcaaccg cggccgcacc      1080
gagtgggcga agaagccac ccgcatggac tacaaggacc acgacggcga ctacaaggac      1140
cacgacatcg actacaagga cgacgacgac aagtga 1176
  
```

<210> SEQ ID NO 41
 <211> LENGTH: 1176
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polynucleotide

<400> SEQUENCE: 41

```

atggccaccg catccacttt ctggcggttc aatgcccgtc gggcgacct gcgtcgctcg      60
gggggctcgg ggccccggcg cccagcgagg cccctccccg tgcgeggggcg cgccatcccc      120
ccccgcatca tcgtggtgtc ctctctctcc tccaaggtga acccctgaa gaccgaggcc      180
gtggtgtcct ccggcctggc cgaccgctg cgcctgggct ccctgaccga ggacggcctg      240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaacaa gaccgccacc      300
gtggagacca tcgccaacct gctgcaggag gtgggctgca accacgcccc gtcctgtggc      360
tactccaccg tggccttctc caccaccccc accatgcgca agctgcgctc gatctgggtg      420
accgcccgca tgcacatcga gatctacaag taccgccctc ggtccgacgt ggtggagatc      480
  
```

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gagtccctggg gccagggcga gggcaagatc ggcttccgcc gcgactggat cctgcgcgac 540
tacgccaccg gccaggtgat cggcccgccc acctccaagt gggatgatgat gaaccaggac 600
acccgccgcc tgcagaaggt ggacgtggac gtgcccgcac agtacctggt gcaactgccc 660
cgcgagctgc gcctggcctt ccccaggag aacaactcct ccctgaagaa gatctccaag 720
ctggaggacc cctcccagta ctccaagctg ggcttgggtc cccgccgcgc cgacctggac 780
atgaaccagc acgtgaacaa cgtgacctac atcggctggg tgctggagtc catgccccag 840
gagatcatcg acaccacga gctgcagacc atcacctgg actaccgcc cgagtgccag 900
cacgacgacg tgggtgactc cctgacctc cccgagcct cggaggacgc cgaggccgtg 960
ttcaaccaca acggcaccaa cggctccgcc aacgtgtccg ccaacgacca cggctgccgc 1020
aacttcctgc acctgctgcg cctgtccggc aacggcctgg agatcaaccg cggccgcacc 1080
gagtggcgca agaagccac ccgcatggac tacaaggacc acgacggcga ctacaaggac 1140
cacgacatcg actacaagga cgacgacgac aagtga 1176

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<210> SEQ ID NO 42
<211> LENGTH: 1176
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

```

```

<400> SEQUENCE: 42
atggccaccg catccacttt ctccgcttc aatgcccgtc gcggcgacct gcgtcgtctg 60
gcgggctccg ggcgccggcg cccagcaggg cccctccccg tgcgcccggc cgccatcccc 120
cccccatca tcgtggtgtc ctctctctcc tccaaggatga acccctgaa gaccgaggcc 180
gtggtgtcct ccggcctggc cgaccgctg cgcctgggtc ccctgaccga ggacggctg 240
tcctacaagg agaagtcat cgtgcgctgc tacgaggtgg gcatcaacaa gaccgccacc 300
gtggagacca tcgccaacct gctgcaggag gtgggctgca accacgccc gtcctggggc 360
tactccaccg gcggttctc caccacccc accatgcgca agctgcgct gatctgggtg 420
accgccgca tgcacatoga gatctacaag taccgccct ggtccgacgt ggtggagatc 480
gagtccctggg gccagggcga gggcaagatc ggcccgcgcc gcgactggat cctgcgcgac 540
tacgccaccg gccaggtgat cggcccgccc acctccaagt gggatgatgat gaaccaggac 600
acccgccgcc tgcagaaggt ggacgtggac gtgcccgcac agtacctggt gcaactgccc 660
cgcgagctgc gcctggcctt ccccaggag aacaactcct ccctgaagaa gatctccaag 720
ctggaggacc cctcccagta ctccaagctg ggcttgggtc cccgccgcgc cgacctggac 780
atgaaccagc acgtgaacaa cgtgacctac atcggctggg tgctggagtc catgccccag 840
gagatcatcg acaccacga gctgcagacc atcacctgg actaccgcc cgagtgccag 900
cacgacgacg tgggtgactc cctgacctc cccgagcct cggaggacgc cgaggccgtg 960
ttcaaccaca acggcaccaa cggctccgcc aacgtgtccg ccaacgacca cggctgccgc 1020
aacttcctgc acctgctgcg cctgtccggc aacggcctgg agatcaaccg cggccgcacc 1080
gagtggcgca agaagccac ccgcatggac tacaaggacc acgacggcga ctacaaggac 1140
cacgacatcg actacaagga cgacgacgac aagtga 1176

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<210> SEQ ID NO 43
<211> LENGTH: 1176

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

<400> SEQUENCE: 43
atggccaccg catccacttt ctggcggttc aatgcccgtc gcggcgacct gcgtcgctcg      60
gctggctccg gggcccggcg cccagcgagg cccctccccg tggcgggcg cgccatcccc      120
ccccgcatca tcgtggtgtc ctctcctcc tccaaggtga acccctgaa gaccgaggcc      180
gtggtgtcct ccggcctggc cgaccgcctg cgcctgggct ccctgaccga ggacggcctg      240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaaaaa gaccgccacc      300
gtggagacca tcgccaacct gctgcaggag gtgggctgca accacgccc gtcctggggc      360
tactccaccg gcggttctc caccaccccc accatgcgca agctgcgctc gatctgggtg      420
accgcccgca tgcacatcga gatctacaag taccgccctt ggtccgacgt ggtggagatc      480
gagtcctggg gccagggcga gggcaagatc ggcaagcgc gcgactggat cctgcgcgac      540
tacgccaccg gccaggtgat cggccgcgcc acctccaagt gggatgatgat gaaccaggac      600
accgcccgcc tgcagaaggt ggacgtggac gtgcgcgacg agtacctggt gcaactcccc      660
cgcgagctgc gcctggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag      720
ctggaggacc cctcccagta ctccaagctg ggcctgggtc cccgcccgcg cgacctggac      780
atgaaccagc acgtgaacaa cgtgacctac atcggctggg tgetggagtc catgcccag      840
gagatcatcg acaccacga gctgcagacc atcacctgg actaccgccg cgagtgccag      900
cacgacgacg tggtgactc cctgacctc cccgagcctt ccgaggacgc cgaggccgtg      960
ttcaaccaca acggcaccaa cggctccgcc aacgtgtccg ccaacgacca cggctgcgcg      1020
aacttcctgc acctgctgcg cctgtccggc aacggcctgg agatcaaccg cggcccacc      1080
gagtgggcga agaagccac ccgcatggac tacaaggacc acgacggcga ctacaaggac      1140
cacgacatcg actacaagga cgacgacgac aagtga      1176

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<210> SEQ ID NO 44
<211> LENGTH: 1176
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

<400> SEQUENCE: 44
atggccaccg catccacttt ctggcggttc aatgcccgtc gcggcgacct gcgtcgctcg      60
gctggctccg gggcccggcg cccagcgagg cccctccccg tggcgggcg cgccatcccc      120
ccccgcatca tcgtggtgtc ctctcctcc tccaaggtga acccctgaa gaccgaggcc      180
gtggtgtcct ccggcctggc cgaccgcctg cgcctgggct ccctgaccga ggacggcctg      240
tcctacaagg agaagttcat cgtgcgctgc tacgaggtgg gcatcaaaaa gaccgccacc      300
gtggagacca tcgccaacct gctgcaggag gtgggctgca accacgccc gtcctggggc      360
tactccaccg gcggttctc caccaccccc accatgcgca agctgcgctc gatctgggtg      420
accgcccgca tgcacatcga gatctacaag taccgccctt ggtccgacgt ggtggagatc      480
gagtcctggg gccagggcga gggcaagatc ggcaagcgc gcgactggat cctgcgcgac      540
tacgccaccg gccaggtgat cggccgcgcc acctccaagt gggatgatgat gaaccaggac      600

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acccgccgcc tgcagaaggt ggacgtggac gtgcgcgacg agtacctggt gcactgcccc 660
cgcgagctgc gcctggcctt ccccgaggag aacaactcct ccctgaagaa gatctccaag 720
ctggaggacc cctcccagta ctccaagctg ggctgtgtgc cccgccgcgc cgacctggac 780
atgaaccagc acgtgaacaa cgtgacctac atcggtgtgg tgctggagtc catgccccag 840
gagatcatcg acaccacga gctgcagacc atcacctctg actaccgccg cgagtgcacg 900
cacgacgacg tgggtgactc cctgacctcc cccgagccct cggaggacgc cgaggccgtg 960
ttcaaccaca acggcaccaa cggtccgcc aacgtgtccg ccaacgacca cggtgcccgc 1020
aacttcctgc acctgctgcg cctgtccggc aacggcctgg agatcaaccg cggccgcacc 1080
gagtggcgca agaagccac ccgcatggac tacaaggacc acgacggcga ctacaaggac 1140
cacgacatcg actacaagga cgacgacgac aagtga 1176

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<210> SEQ ID NO 45

<211> LENGTH: 330

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 45

```

Ile Pro Pro Arg Ile Ile Val Val Ser Ser Ser Ser Ser Lys Val Asn
1           5           10           15
Pro Leu Lys Thr Glu Ala Val Val Ser Ser Gly Leu Ala Asp Arg Leu
20           25           30
Arg Leu Gly Ser Leu Thr Glu Asp Gly Leu Ser Tyr Lys Glu Lys Phe
35           40           45
Ile Val Arg Cys Tyr Glu Val Gly Ile Asn Lys Thr Ala Thr Val Glu
50           55           60
Thr Ile Ala Asn Leu Leu Gln Glu Val Gly Cys Asn His Ala Gln Ser
65           70           75           80
Val Gly Tyr Ser Thr Gly Gly Phe Ser Thr Thr Pro Thr Met Arg Lys
85           90           95
Leu Arg Leu Ile Trp Val Thr Ala Arg Met His Ile Glu Ile Tyr Lys
100          105          110
Tyr Pro Ala Trp Ser Asp Val Val Glu Ile Glu Ser Trp Gly Gln Gly
115          120          125
Glu Gly Lys Ile Gly Thr Arg Arg Asp Trp Ile Leu Arg Asp Tyr Ala
130          135          140
Thr Gly Gln Val Ile Gly Arg Ala Thr Ser Lys Trp Val Met Met Asn
145          150          155          160
Gln Asp Thr Arg Arg Leu Gln Lys Val Asp Val Asp Val Arg Asp Glu
165          170          175
Tyr Leu Val His Cys Pro Arg Glu Leu Arg Leu Ala Phe Pro Glu Glu
180          185          190
Asn Asn Ser Ser Leu Lys Lys Ile Ser Lys Leu Glu Asp Pro Ser Gln
195          200          205
Tyr Ser Lys Leu Gly Leu Val Pro Arg Arg Ala Asp Leu Asp Met Asn
210          215          220
Gln His Val Asn Asn Val Thr Tyr Ile Gly Trp Val Leu Glu Ser Met
225          230          235          240
Pro Gln Glu Ile Ile Asp Thr His Glu Leu Gln Thr Ile Thr Leu Asp
245          250          255

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Tyr Arg Arg Glu Cys Gln His Asp Asp Val Val Asp Ser Leu Thr Ser
 260 265 270

Pro Glu Pro Ser Glu Asp Ala Glu Ala Val Phe Asn His Asn Gly Thr
 275 280 285

Asn Gly Ser Ala Asn Val Ser Ala Asn Asp His Gly Cys Arg Asn Phe
 290 295 300

Leu His Leu Leu Arg Leu Ser Gly Asn Gly Leu Glu Ile Asn Arg Gly
 305 310 315 320

Arg Thr Glu Trp Arg Lys Lys Pro Thr Arg
 325 330

<210> SEQ ID NO 46
 <211> LENGTH: 8510
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 46

```

gctcttccca actcagataa taccaatacc cctccttctc ctctcatcc attcagtacc      60
cccccccttc tcttcccaaa gcagcaagcg cgtggcttac agaagaacaa tcgggttccg    120
ccaaagtgcg cgagcactgc ccgacggcgg cgcgcccagc agcccgttg gccacacagg    180
caacgaatac attcaatagg gggcctcgca gaatggaagg agcggtaaag ggtacaggag    240
cactgcgcac aaggggctg tgcaggagtg actgactggg cgggcagacg gcgcacccgcg    300
ggcgcaggca agcagggaaag attgaagcgg cagggaggag gatgctgatt gaggggggca    360
tcgcagtctc tcttggcccc gggataagga agcaaatatt cggccggttg ggttgtgtgt    420
gtgcacgttt tcttcttcag agtctgtggg gtgcttccag ggaggatata agcagcagga    480
tcgaatcccg cgaccagcgt tccccatcc agccaaccac cctgtcggta ccctttcttg    540
cgctatgaca cttccagcaa aaggtagggc gggctgcgag acggcttccc ggcgctgcat    600
gcaacaccga tgatgcttcg acccccggaa gctccttcgg ggctgcatgg gcgctccgat    660
gccgctccag ggcgagcgcg gtttaaatag ccaggcccc gattgcaaag acattatagc    720
gagctaccaa agccatattc aaacacctag atcactacca cttctacaca ggccactcga    780
gcttgtgatc gcactccgct aagggggcgc ctcttctct tctgttcagt cacaaccgcg    840
aaactctaga atatcaatgc tgctgcagge cttcctgttc ctgctggccg gcttcgccgc    900
caagatcagc gcctccatga cgaacgagac gtccgaccgc cccctggtgc acttcacccc    960
caacaagggc tggatgaacg accccaacgg cctgtggtac gacgagaagg acgccaagtg   1020
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ccacgccacg tccgacgacc tgaccaactg ggaggaccag cccatcgcca tcgccccgaa   1140
gcgcaacgac tccggcgccg tctccggctc catggtgggtg gactacaaca acacctccgg   1200
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cgagatctac tcctccgacg acctgaagtc ctggaagctg gagtccgctg tcgccaacga   1500
gggcttctc ggetaccagt acgagtgcc cggcctgac gaggtcccca ccgagcagga   1560
ccccagcaag tcctactggg tgatgttcat ctccatcaac cccggcgccc cgccggcggg   1620
    
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ctccttcaac	cagtacttcg	tcggcagctt	caacggcacc	cacttcgagg	ccttcgacaa	1680
ccagteccgc	gtggtggact	tcggcaagga	ctactacgcc	ctgcagacct	tcttcaaac	1740
cgaccocgacc	tacgggagcg	ccctgggcat	cgcgtgggcc	tccaactggg	agtactccgc	1800
cttcgtgccc	accaacecct	ggcgtcctc	catgtccctc	gtgcgcaagt	tctccctcaa	1860
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caacagctac	aacgtcgacc	tgtccaacag	caccggcacc	ctggagttcg	agctgggtga	2040
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gctgatctgg	ctggtggact	ggtgggcccg	cgtgaagatc	aaggtgttca	tgacccccga	3840
gtccttcaac	ctgatgggca	aggagcacgc	cctgggtggg	gccaaaccacc	gctccgacat	3900
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cgtgatgaag aagtcccca agttctgcc cgtgatcggc tggccatgt ggttctccga	4020
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gcgcctgaag gacttccccc gcccttctg gctggccttc ttegtggagg gcaccgctt	4140
caccagggcc aagttcttgg ccgcccagga gtacgcgcc tcccagggcc tgcccatccc	4200
ccgcaacgtg ctgatcccc gcaccaaggg ctctcgtgtcc gccgtgtccc acatgcgctc	4260
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gatgaaggag ctgcccagaga ccgacgaggc cgtggcccag tgggtcaagg acatgttcgt	4440
ggagaaggac aagctgctgg acaagcacat ccgagaggac accttctccg accagcccat	4500
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tcgacaaacta gtatggccac cgcacccact ttctcggcgt tcaatgcccc ctgcccgcac	6240
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aagaccgagg ccgtggtgtc ctcggcctg gccgaaccgcc tgegctggg ctcctgacc 6420
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cagtccgtgg gctactccac cgccggcttc tccaccacc ccaccatgcg caagctgcg 6600
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ggaccgcatg atccaccgga aaagcgcacg cgctggagcg cgcagaggac cacagagaag 8340
cggaaagagac gccagtactg gcaagcaggc tggtegggtc catggcgcg tactaccctc 8400
gctatgactc gggctcctcg ccgctggcg gtgctgacaa ttcgtttagt ggagcagcga 8460
ctccattcag ctaccagtcg aactcagtg caccagtact cccgctcttc 8510

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<210> SEQ ID NO 47

<211> LENGTH: 1188

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 47

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actagtatgg ccaccgcatc cactttctcg gcgttcaatg cccgctgcgg cgacctgcgt    60
cgctcggcgg gctccggggc ccggcgccca gcgaggcccc tccccgtgcg cgggcgcgcc    120
atcccccccc gcatcatcgt ggtgtcctcc tcctcctcca aggtgaaccc cctgaagacc    180
gaggccgtgg tgtcctccgg cctggccgac cgcctgcgcc tgggctccct gaccgaggac    240
ggcctgtcct acaaggagaa gttcatcgtg cgctgctacg aggtgggcat caacaagacc    300
gccaccgtgg agaccatcgc caacctgctg caggaggtgg gctgcaacca cgcccagtcc    360
gtgggctact ccaccgcggg cttctccacc acccccacca tgcgcaagct gcgcctgatc    420
tgggtgaccg cccgcatgca catcgagatc tacaagtacc ccgcctggtc cgacgtggtg    480
gagatcgagt cctggggcca gggcgagggc aagatcgcca cccgcccgca ctggatcctg    540
cgcgactacg ccaccggcca ggtgatcggc cgcgccacct ccaagtgggt gatgatgaac    600
caggacaccc gccgcctgca gaaggtggac gtggacgtgc gcgacgagta cctggtgcac    660
tgcccccgcg agctgcgcct ggccttcccc gaggagaaca actcctccct gaagaagatc    720
tccaagctgg aggacccctc ccagtactcc aagctggggc tggtgccccg ccgcgccgac    780
ctggacatga accagcacgt gaacaacgtg acctacatcg gctgggtgct ggagtccatg    840
ccccaggaga tcatcgacac ccacgagctg cagaccatca ccctggacta ccgccgcgag    900
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gccgtgttca accacaacgg caccaacggc tccgccaacg tgtccgcca cgaccacggc    1020
tgccgcaact tcctgcacct gctgcgcctg tccgcaacg gcctggagat caaccgcggc    1080
cgcaccgagt ggcgcaagaa gccaccgcc cctggactaca aggaccacga cggcgactac    1140
aaggaccacg acatcgacta caaggacgac gacgacaagt gaatcgat    1188

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<210> SEQ ID NO 48

<211> LENGTH: 1188

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 48

```

actagtatgg ccaccgcatc cactttctcg gcgttcaatg cccgctgcgg cgacctgcgt    60
cgctcggcgg gctccggggc ccggcgccca gcgaggcccc tccccgtgcg cgggcgcgcc    120
atcccccccc gcatcatcgt ggtgtcctcc tcctcctcca aggtgaaccc cctgaagacc    180
gaggccgtgg tgtcctccgg cctggccgac cgcctgcgcc tgggctccct gaccgaggac    240
ggcctgtcct acaaggagaa gttcatcgtg cgctgctacg aggtgggcat caacaagacc    300
gccaccgtgg agaccatcgc caacctgctg caggaggtgg cgtgcaacca cgcccagtcc    360
gtgggctact ccaccgcggg cttcgccacc acccccacca tgcgcaagct gcgcctgatc    420
tgggtgaccg cccgcatgca catcgagatc tacaagtacc ccgcctggtc cgacgtggtg    480
gagatcgagt cctggggcca gggcgagggc aagatcgcca cccgcccgca ctggatcctg    540
cgcgactacg ccaccggcca ggtgatcggc cgcgccacct ccaagtgggt gatgatgaac    600
caggacaccc gccgcctgca gaaggtggac gtggacgtgc gcgacgagta cctggtgcac    660
tgcccccgcg agctgcgcct ggccttcccc gaggagaaca actcctccct gaagaagatc    720

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tccaagctgg aggaccctc ccagtactcc aagctgggcc tggcgcccc cgcgccgac 780
ctggacatga accagcacgt gaacaacgtg acctacatcg gctgggtgct ggagtccatg 840
ccccaggaga tcatcgacac ccacgagctg cagaccatca ccctggacta ccgcccgag 900
tgccagcacg acgacgtggt ggactcctg acctccccg agccctccga ggacgccgag 960
gccgtgttca accacaacgg caccaacggc tccgccaacg tgtccgcaa cgaccacggc 1020
tgccgcaact tcctgcacct gctgcgcctg tccgcaacg gcctggagat caaccgaggc 1080
cgcaccgagt ggcgcaagaa gccaccgcc atggactaca aggaccaga cggcgactac 1140
aaggaccaag acatcgacta caaggacgac gacgacaagt gaatcgat 1188

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<210> SEQ ID NO 49
<211> LENGTH: 1188
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

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<400> SEQUENCE: 49

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actagtatgg ccaccgcatc cactttctcg gcgttcaatg cccgctgagg cgacctgctg 60
cgctcggcgg gctccggggc ccggcgccca gcgaggcccc tcccctgctg cgggcgcgcc 120
atcccccccc gcatcatcgt ggtgtctctc tctctctcca aggtgaaccc cctgaagacc 180
gaggccgtgg tgctctccgg cctggccgac cgcctgcgcc tgggctcctt gaccgaggac 240
ggcctgtcct acaaggagaa gttcatcgtg cgctgctacg aggtgggcat caacaagacc 300
gccaccgtgg agaccatcgc caacctgctg caggagggtg cgtgcaacca cgcccagtcc 360
gtgggctact ccaccggcgg cttctccacc acccccacca tgcgcaagct gcgcctgatc 420
tgggtgaccg cccgcatgca catcgagatc tacaagtacc ccgcctggtc cgacgtggtg 480
gagatcgagt cctggggcca gggcgagggc aagatcggca cccgccgca ctggatcctg 540
cgcgactacg ccaccggcca ggtgatcggc cgcgccacct ccaagtgggt gatgatgaac 600
caggacaccc gccgcctgca gaaggtggac gcggacgtgc gcgacgagta cctggtgcac 660
tgcccccgcg agctgcgcct ggccctcccc gaggagaaca actcctcctt gaagaagatc 720
tccaagctgg aggaccctc ccagtactcc aagctgggcc tggcgcccc cgcgccgac 780
ctggacatga accagcacgt gaacaacgtg acctacatcg gctgggtgct ggagtccatg 840
ccccaggaga tcatcgacac ccacgagctg cagaccatca ccctggacta ccgcccgag 900
tgccagcacg acgacgtggt ggactcctg acctccccg agccctccga ggacgccgag 960
gccgtgttca accacaacgg caccaacggc tccgccaacg tgtccgcaa cgaccacggc 1020
tgccgcaact tcctgcacct gctgcgcctg tccgcaacg gcctggagat caaccgaggc 1080
cgcaccgagt ggcgcaagaa gccaccgcc atggactaca aggaccaga cggcgactac 1140
aaggaccaag acatcgacta caaggacgac gacgacaagt gaatcgat 1188

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<210> SEQ ID NO 50
<211> LENGTH: 1188
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

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<400> SEQUENCE: 50

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actagtatgg ccaccgcata cactttctcg gcgttcaatg cccgctgcgg cgacctgcgt	60
cgctcggcgg gctccgggcc ccggcgccca gcgaggcccc tccccgtgcg cgggcgcgcc	120
atcccccccc gcatcatcgt ggtgtcctcc tctctctcca aggtgaaccc cctgaagacc	180
gaggccgtgg tgtcctccgg cctggccgac cgctcgcgcc tgggctccct gaccgaggac	240
ggcctgtcct acaaggagaa gttcatcgtg cgctgctacg aggtgggcat caacaagacc	300
gccaccgtgg agaccatcgc caacctgctg caggagggtg gctgcaacca cggccagtcc	360
gtgggctact ccaccgcggg cttctccacc acccccacca tgcgcaagct gcgctgatc	420
tgggtgaccg cccgcatgca catcgagatc tacaagtacc ccgctggtc cgactgggtg	480
gagatcgagt cctggggcca gggcgagggc aagatcgcca cccgcccga ctggatcctg	540
cgcgactacg ccaccggcca ggtgatcggc cgcccccact ccaagtgggt gatgatgaac	600
caggacaccc gccgcctgca gaaggtggac gtggacgtgc gcgacgagta cctggtgcac	660
tgcccccgcg agctgcgcct ggccttcccc gaggagaaca actcctccct gaagaagatc	720
tccaagctgg aggaccctc ccagtaactc aagctgggcc tggtgccccg ccgcccgcac	780
ctggacatga accagcacgt gaacaacgtg acctacatcg gctgggtgct ggagtccatg	840
ccccaggaga tcatcgacac ccacgagctg cagaccatca ccctggacta ccgcccgcag	900
tgccagcaag acgacgtggt ggactcctg acctccccg agccctccga ggacgcccag	960
gccgtgttca accacaacgg caccaacggc tccgccaacg tgtccgcca cgaccacggc	1020
tgcgcgaact tctgcaact gctgcgcctg tccgcaacg gcctggagat caaccgccc	1080
cgcaccgagt ggcgcaagaa gccaccgcc atggactaca aggaccaga cggcgactac	1140
aaggaccacg acatcgacta caaggacgac gacgacaagt gaatcgat	1188

<210> SEQ ID NO 51

<211> LENGTH: 1188

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 51

actagtatgg ccaccgcata cactttctcg gcgttcaatg cccgctgcgg cgacctgcgt	60
cgctcggcgg gctccgggcc ccggcgccca gcgaggcccc tccccgtgcg cgggcgcgcc	120
atcccccccc gcatcatcgt ggtgtcctcc tctctctcca aggtgaaccc cctgaagacc	180
gaggccgtgg tgtcctccgg cctggccgac cgctcgcgcc tgggctccct gaccgaggac	240
ggcctgtcct acaaggagaa gttcatcgtg cgctgctacg aggtgggcat caacaagacc	300
gccaccgtgg agaccatcgc caacctgctg caggagggtg gctgcaacca cggccagtcc	360
gtgggctact ccaccgcggg cttctccacc acccccacca tgcgcaagct gcgctgatc	420
tgggtgaccg cccgcatgca catcgagatc tacaagtacc ccgctggtc cgactgggtg	480
gagatcgagt cctggggcca gggcgagggc aagatcgcca cccgcccga ctggatcctg	540
cgcgactacg ccaccggcca ggtgatcggc cgcccccact ccaagtgggt gatgatgaac	600
caggacaccc gccgcctgca gaaggtggac gcggacgtgc gcgacgagta cctggtgcac	660
tgcccccgcg agctgcgcct ggccttcccc gaggagaaca actcctccct gaagaagatc	720
tccaagctgg aggaccctc ccagtaactc aagctgggcc tggtgccccg ccgcccgcac	780
ctggacatga accagcacgt gaacaacgtg acctacatcg gctgggtgct ggagtccatg	840

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ccccaggaga tcatcgacac ccacgagctg cagaccatca ccctggacta ccgccgcgag  900
tgccagcacg acgacgtggt ggactccctg acctcccccg agccctccga ggacgccgag  960
gccgtgttca accacaacgg caccaacggc tccgccaacg tgtccgcca cgaccacggc  1020
tgccgcaact tcctgcaact gctgcgctg tccggcaacg gcctggagat caaccgcggc  1080
cgcaccgagt ggcgcaagaa gccaccaccg atggactaca aggaccacga cggcgactac  1140
aaggaccacg acatcgacta caaggacgac gacgacaagt gaatcgat  1188

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<210> SEQ ID NO 52
<211> LENGTH: 1188
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
                             polynucleotide

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<400> SEQUENCE: 52
actagtatgg ccaccgcac cactttctcg gcgttcaatg cccgctgcgg cgacctgcgt  60
cgctcggcgg gctccgggccc ccggcgccca gcgaggcccc tccccgtgcg cgggcgcgcc  120
atcccccccc gcatcatcgt ggtgtcctcc tcctcctcca aggtgaaccc cctgaagacc  180
gaggccgtgg tgtcctccgg cctggcggac cgcctgcgcc tgggctccct gaccgaggac  240
ggcctgtcct acaaggagaa gttcatcgtg cgctgctacg aggtgggcat caacaagacc  300
gccaccgtgg agaccatcgc caacctgctg caggaggtgg cgtgcaacca cgcccagtcc  360
gtgggctact ccaccgcggc ctcgcgccac acccccacca tgcgcaagct gcgcctgatc  420
tgggtgacgg cccgcacgca catcgagatc tacaagtacc ccgcctggtc cgacgtggtg  480
gagatcgagt cctggggcca gggcgagggc aagatcgcca cccgcccgca ctggatcctg  540
cgcgactacg ccaccggcca ggtgatcggc cgcgccacct ccaagtgggt gatgatgaac  600
caggacaccc gccgcctgca gaaggtggac gtggacgtgc gcgacgagta cctggtgcac  660
tgcccccgcg agctgcgctt ggccttcccc gaggagaaca actcctccct gaagaagatc  720
tccaagctgg aggaccctc ccagtactcc aagctgggccc tggtgccccg ccgcgccgac  780
ctggacatga accagcacgt gaacaacgtg acctacatcg gctgggtgct ggagtccatg  840
ccccaggaga tcatcgacac ccacgagctg cagaccatca ccctggacta ccgccgcgag  900
tgccagcacg acgacgtggt ggactccctg acctcccccg agccctccga ggacgccgag  960
gccgtgttca accacaacgg caccaacggc tccgccaacg tgtccgcca cgaccacggc  1020
tgccgcaact tcctgcaact gctgcgctg tccggcaacg gcctggagat caaccgcggc  1080
cgcaccgagt ggcgcaagaa gccaccaccg atggactaca aggaccacga cggcgactac  1140
aaggaccacg acatcgacta caaggacgac gacgacaagt gaatcgat  1188

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<210> SEQ ID NO 53
<211> LENGTH: 1188
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
                             polynucleotide

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<400> SEQUENCE: 53
actagtatgg ccaccgcac cactttctcg gcgttcaatg cccgctgcgg cgacctgcgt  60
cgctcggcgg gctccgggccc ccggcgccca gcgaggcccc tccccgtgcg cgggcgcgcc  120
atcccccccc gcatcatcgt ggtgtcctcc tcctcctcca aggtgaaccc cctgaagacc  180

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gaggccgtgg	tgtctccgg	cctggccgac	cgctgcgcc	tgggtccct	gaccgaggac	240
ggcctgtcct	acaaggagaa	gttcatcgtg	cgctgctacg	aggtgggcat	caacaagacc	300
gccaccgtgg	agaccatcgc	caacctgctg	caggagggtg	cggtcaacca	cgcccagtcc	360
gtgggctact	ccaccgccgg	cttctccacc	acccccacca	tgcgcaagct	gcgcctgate	420
tgggtgaccg	cccgcacgca	catcgagatc	tacaagtacc	ccgcctggtc	cgacgtggtg	480
gagatcgagt	cctggggcca	gggcgagggc	aagatcgcca	cccgcgcga	ctggatcctg	540
cgcgactacg	ccaccggcca	ggtgatcggc	cgcgccacct	ccaagtgggt	gatgatgaac	600
caggacaccc	ggcgctgca	gaaggtggac	gcggaagtgc	gcgacgagta	cctggtgcac	660
tgcccccgcg	agctgcgcct	ggccttcccc	gaggagaaca	actcctccct	gaagaagatc	720
tccaagctgg	aggaccctc	ccagtactcc	aagctgggcc	tggtgccccg	cgcgccgac	780
ctggacatga	accagcacgt	gaacaacgtg	acctacatcg	gctgggtgct	ggagtccatg	840
ccccaggaga	tcatcgacac	ccacgagctg	cagaccatca	ccctggacta	cgcccgcgag	900
tgccagcacg	acgacgtggt	ggactccctg	acctcccccg	agccctccga	ggacgcccag	960
gccgtgttca	accacaacgg	caccaacggc	tccgccaacg	tgtccgcca	cgaccacggc	1020
tgccgcaact	tcctgcacct	gctgcgcctg	tccgccaacg	gcctggagat	caaccgcggc	1080
cgaccgaggt	ggcgcaagaa	gccccccgc	atggactaca	aggaccacga	cgcgactac	1140
aaggaccacg	acatcgacta	caaggacgac	gacgacaagt	gaatcgat		1188

<210> SEQ ID NO 54

<211> LENGTH: 1188

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 54

actagtatgg	ccaccgcatc	cactttctcg	gcgttcaatg	cccgtgcgg	cgacctgcgt	60
cgctcggcgg	gctccgggcc	ccggcgcca	gcgaggcccc	tcccctgctg	cgggcgcgcc	120
atccccccc	gcatcatcgt	ggtgtcctcc	tctctctcca	aggtgaaccc	cctgaagacc	180
gaggccgtgg	tgtctccgg	cctggccgac	cgctgcgcc	tgggtccct	gaccgaggac	240
ggcctgtcct	acaaggagaa	gttcatcgtg	cgctgctacg	aggtgggcat	caacaagacc	300
gccaccgtgg	agaccatcgc	caacctgctg	caggagggtg	cggtcaacca	cgcccagtcc	360
gtgggctact	ccaccgccgg	cttctccacc	acccccacca	tgcgcaagct	gcgcctgate	420
tgggtgaccg	cccgcacgca	catcgagatc	tacaagtacc	ccgcctggtc	cgacgtggtg	480
gagatcgagt	cctggggcca	gggcgagggc	aagatcgcca	cccgcgcga	ctggatcctg	540
cgcgactacg	ccaccggcca	ggtgatcggc	cgcgccacct	ccaagtgggt	gatgatgaac	600
caggacaccc	ggcgctgca	gaaggtggac	gcggaagtgc	gcgacgagta	cctggtgcac	660
tgcccccgcg	agctgcgcct	ggccttcccc	gaggagaaca	actcctccct	gaagaagatc	720
tccaagctgg	aggaccctc	ccagtactcc	aagctgggcc	tggtgccccg	cgcgccgac	780
ctggacatga	accagcacgt	gaacaacgtg	acctacatcg	gctgggtgct	ggagtccatg	840
ccccaggaga	tcatcgacac	ccacgagctg	cagaccatca	ccctggacta	cgcccgcgag	900
tgccagcacg	acgacgtggt	ggactccctg	acctcccccg	agccctccga	ggacgcccag	960
gccgtgttca	accacaacgg	caccaacggc	tccgccaacg	tgtccgcca	cgaccacggc	1020

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tgccgcaact tcctgcaact gctgcgctg tccggcaacg gcctggagat caaccgcggc 1080
cgcaccgagt ggcgcaagaa gcccaccgc atggactaca aggaccacga cggcgactac 1140
aaggaccacg acatcgacta caaggacgac gacgacaagt gaatcgat 1188

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<210> SEQ ID NO 55
<211> LENGTH: 1188
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

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<400> SEQUENCE: 55

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actagtatgg ccaccgcata cactttctcg gcgttcaatg cccgctgagg cgacctgagt 60
cgctcggcgg gctcggggcc ccggcgccca gcgaggcccc tccccgtgag cgggcgagcc 120
atcccccccc gcatcatcgt ggtgtcctcc tcctcctcca aggtgaaccc cctgaagacc 180
gaggccgtgg tgtcctccgg cctggccgac cgcctgagcc tgggctccct gaccgaggac 240
ggcctgtcct acaaggagaa gttcatcgtg cgctgctacg aggtgggcat caacaagacc 300
gccaccgtgg agaccatcgc caacctgctg caggagggtg gctgcaacca cggccagtcc 360
gtgggctact ccaccgcagg cttcgccacc acccccacca tgcgcaagct gcgctgatc 420
tgggtgaccg cccgcatgca catcgagatc tacaagtacc ccgctggctc cgactgggtg 480
gagatcgagt cctggggcca gggcgagggc aagatcgcca cccgcccga ctggatcctg 540
cgcgactacg ccaccggcca ggtgatcggc cgcgccacct ccaagtgggt gatgatgaac 600
caggacaccc gccgcctgca gaaggtggac gcggacgtgc gcgacagta cctggtgcac 660
tgcccccgag agctgagcct ggccctcccc gaggagaaca actcctccct gaagaagatc 720
tccaagctgg aggaccctc ccagtaactc aagctgggcc tggtgccccg ccgcccagac 780
ctggacatga accagcacgt gaacaacgtg acctacatcg gctgggtgct ggagtccatg 840
ccccaggaga tcatcgacac ccacgagctg cagaccatca ccctggacta ccgcccagag 900
tgccagcacg acgacgtggt ggactcctg acctcccccg agccctccga ggacgcccag 960
gccgtgttca accacaacgg caccaacggc tccgccaacg tgtccgcca cgaccacggc 1020
tgccgcaact tcctgcaact gctgcgctg tccggcaacg gcctggagat caaccgcggc 1080
cgcaccgagt ggcgcaagaa gcccaccgc atggactaca aggaccacga cggcgactac 1140
aaggaccacg acatcgacta caaggacgac gacgacaagt gaatcgat 1188

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<210> SEQ ID NO 56
<211> LENGTH: 1188
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

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<400> SEQUENCE: 56

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actagtatgg ccaccgcata cactttctcg gcgttcaatg cccgctgagg cgacctgagt 60
cgctcggcgg gctcggggcc ccggcgccca gcgaggcccc tccccgtgag cgggcgagcc 120
atcccccccc gcatcatcgt ggtgtcctcc tcctcctcca aggtgaaccc cctgaagacc 180
gaggccgtgg tgtcctccgg cctggccgac cgcctgagcc tgggctccct gaccgaggac 240
ggcctgtcct acaaggagaa gttcatcgtg cgctgctacg aggtgggcat caacaagacc 300

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gccaccgtgg agaccatcgc caacctgctg caggagggtg cgtgcaacca cgcccagtcc 360
gtgggctact ccaccgcggt cttcgccacc acccccacca tgcgcaagct gcgcctgatc 420
tgggtgacgg cccgcacgca catcgagatc tacaagtacc ccgcctggtc cgacgtggtg 480
gagatcgagt cctggggcca gggcgagggc aagatcgcca cccgcgcga ctggatcctg 540
cgcgactacg ccaccggcca ggtgatcggc cgcgccacct ccaagtgggt gatgatgaac 600
caggacaccc gccgcctgca gaaggtggac gcggacgtgc gcgacgagta cctggtgcac 660
tgcccccgcg agctgcgect ggccttcccc gaggagaaca actcctcctc gaagaagatc 720
tccaagctgg aggacccctc ccagtactcc aagctgggccc tgggtgcccc cgcgccgac 780
ctggacatga accagcacgt gaacaacgtg acctacatcg gctgggtgct ggagtccatg 840
ccccaggaga tcatcgacac ccacgagctg cagaccatca ccctggacta ccgccgcgag 900
tgccagcacg acgacgtggt ggactccctg acctcccccg agcctcctga ggacgccgag 960
gccgtgttca accacaacgg caccaacggc tccgccaacg tgtccgcaa cgaccacggc 1020
tgccgcaact tcctgcaact gctgcgectg tccggcaacg gcctggagat caaccgcggc 1080
cgcaccgagt ggcgcaagaa gccaccgcc atggactaca aggaccacga cggcgactac 1140
aaggaccacg acatcgacta caaggacgac gacgacaagt gaatcgat 1188

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<210> SEQ ID NO 57
<211> LENGTH: 582
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
    polynucleotide

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<400> SEQUENCE: 57
gaattcgct gctcaagcgg gcgctcaaca tgcagagcgt cagcagacg ggctgtggcg 60
atcgcgagac ggacgaggcc gcctctgcc tgtttgaact gagcgtcagc gctggctaag 120
gggagggaga tcatcccca ggctcgcgcc agggctctga tcccgctcgc ggcggtgatc 180
ggcgcgcatg actacgaccc aacgacgtac gagactgatg tcggtcccga cgaggagcgc 240
cgcgaggcac tcccgggcca ccgaccatgt ttacaccgac cgaaagcact cgctcgtatc 300
cattccgtgc gcccgcaat gcatcatctt ttggtaccga cttcggctct gttttacccc 360
tacgacctgc cttccaaggt gtgagcaact cgcccggaca tgaccgaggg tgatcatccg 420
gatccccagg ccccagcagc cctgcccaga atggetcgcg ctttcagcc tgcaggcccc 480
tctcccaggt cgacgcaacc tacatgacca cccaatctg tcccagacc caaacacct 540
ccttcctgct ttctctgtga tcgctgatca gcaacaacta gt 582

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<210> SEQ ID NO 58
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
    polypeptide

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<400> SEQUENCE: 58

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Met Ala Thr Ala Ser Thr Phe Ser Ala Phe Asn Ala Arg Cys Gly Asp
1           5           10          15
Leu Arg Arg Ser Ala Gly Ser Gly Pro Arg Arg Pro Ala Arg Pro Leu
20          25          30
Pro Val Arg Gly Arg Ala

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1 5 10 15

Val Pro Val Ala Thr Thr Ser Pro Arg
 20 25

<210> SEQ ID NO 69
<211> LENGTH: 72
<212> TYPE: DNA
<213> ORGANISM: *Cuphea hookeriana*
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(72)

<400> SEQUENCE: 69

cac ctg cag gag acc tcc ctg aac cac tgc aag agc acc ggc atc ctg 48
His Leu Gln Glu Thr Ser Leu Asn His Cys Lys Ser Thr Gly Ile Leu
1 5 10 15

ctg gac ggc ttc ggc cgc acc ctg 72
Leu Asp Gly Phe Gly Arg Thr Leu
 20

<210> SEQ ID NO 70
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: *Cuphea hookeriana*

<400> SEQUENCE: 70

His Leu Gln Glu Thr Ser Leu Asn His Cys Lys Ser Thr Gly Ile Leu
1 5 10 15

Leu Asp Gly Phe Gly Arg Thr Leu
 20

<210> SEQ ID NO 71
<211> LENGTH: 72
<212> TYPE: DNA
<213> ORGANISM: *Cuphea avigera*
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(72)

<400> SEQUENCE: 71

tac ctg cag gag acc tcc ctg aac cac tgc aag tcc acc ggc atc ctg 48
Tyr Leu Gln Glu Thr Ser Leu Asn His Cys Lys Ser Thr Gly Ile Leu
1 5 10 15

ctg gac ggc ttc ggc cgc acc ccc 72
Leu Asp Gly Phe Gly Arg Thr Pro
 20

<210> SEQ ID NO 72
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: *Cuphea avigera*

<400> SEQUENCE: 72

Tyr Leu Gln Glu Thr Ser Leu Asn His Cys Lys Ser Thr Gly Ile Leu
1 5 10 15

Leu Asp Gly Phe Gly Arg Thr Pro
 20

<210> SEQ ID NO 73
<211> LENGTH: 82
<212> TYPE: PRT
<213> ORGANISM: *Cuphea palustris*

<400> SEQUENCE: 73

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Lys Arg Asp Leu Ile Trp Val Val Thr Arg Met Lys Ile Met Val Asn
1 5 10 15

Arg Tyr Pro Thr Trp Gly Asp Thr Ile Glu Val Ser Thr Trp Leu Ser
20 25 30

Gln Ser Gly Lys Ile Gly Met Gly Arg Asp Trp Leu Ile Ser Asp Cys
35 40 45

Asn Thr Gly Glu Ile Leu Val Arg Ala Thr Ser Val Tyr Ala Met Met
50 55 60

Asn Gln Lys Thr Arg Arg Phe Ser Lys Leu Pro His Glu Val Arg Gln
65 70 75 80

Glu Phe

<210> SEQ ID NO 74

<211> LENGTH: 82

<212> TYPE: PRT

<213> ORGANISM: *Cuphea hookeriana*

<400> SEQUENCE: 74

Lys Arg Asp Leu Ile Trp Val Val Ile Lys Met Gln Ile Lys Val Asn
1 5 10 15

Arg Tyr Pro Ala Trp Gly Asp Thr Val Glu Ile Asn Thr Arg Phe Ser
20 25 30

Arg Leu Gly Lys Ile Gly Met Gly Arg Asp Trp Leu Ile Ser Asp Cys
35 40 45

Asn Thr Gly Glu Ile Leu Val Arg Ala Thr Ser Ala Tyr Ala Met Met
50 55 60

Asn Gln Lys Thr Arg Arg Leu Ser Lys Leu Pro Tyr Glu Val His Gln
65 70 75 80

Glu Ile

<210> SEQ ID NO 75

<211> LENGTH: 82

<212> TYPE: PRT

<213> ORGANISM: *Cuphea avigera*

<400> SEQUENCE: 75

Lys Arg Asp Leu Ile Trp Val Val Thr Lys Met Lys Ile Lys Val Asn
1 5 10 15

Arg Tyr Pro Ala Trp Gly Asp Thr Val Glu Ile Asn Thr Trp Phe Ser
20 25 30

Arg Leu Gly Lys Ile Gly Lys Gly Arg Asp Trp Leu Ile Ser Asp Cys
35 40 45

Asn Thr Gly Glu Ile Leu Ile Arg Ala Thr Ser Ala Tyr Ala Thr Met
50 55 60

Asn Gln Lys Thr Arg Arg Leu Ser Lys Leu Pro Tyr Glu Val His Gln
65 70 75 80

Glu Ile

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What is claimed is:

1. A non-natural fatty acyl-ACP thioesterase wherein the non-natural thioesterase has at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 1 and comprises:

i) Tyrosine (Y) or phenylalanine (F) at the position corresponding to position 163 of SEQ ID NO: 1 and lysine (K), or alanine (A) at the position corresponding to position 186 of SEQ ID NO: 1; or

ii) Phenylalanine (F) at the position corresponding to position 163 of SEQ ID NO: 1 and/or proline (P), lysine (K), or alanine (A) at the position corresponding to position 186 of SEQ ID NO: 1, wherein the non-natural thioesterase catalyzes the production of increased levels of C8:0 and C10:0 fatty acids in comparison to a wild-type thioesterase.

2. A method for producing a triglyceride oil, the method comprising expressing, in a host cell, the non-natural thioesterase of claim 1; cultivating the host cell; and isolating the oil.

3. A method for increasing the C8 and/or C10 fatty acids in a fatty acid profile of an oil produced by an optionally oleaginous host cell, the method comprising, providing a parent gene encoding a fatty acyl-ACP thioesterase enzyme, mutating the gene to so as to encode a non-natural thioesterase of claim 1; expressing the mutated gene in the host cell; and producing the oil, whereby the C8 and/or C10 fatty acids in the fatty acid profile of the oil are increased.

4. The non-natural fatty acyl-ACP thioesterase of claim 1, further comprising a lysine (K) at the position corresponding to position 228 of SEQ ID NO: 1.

5. A method for producing a triglyceride oil, the method comprising expressing, in a host cell, the non-natural thioesterase of claim 4.

6. The method of claim 3, comprising further mutating the gene so as to encode a non-natural fatty acyl-ACP thio-

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esterase further comprising a lysine (K) at the position corresponding to position 228 of SEQ ID NO: 1.

7. An isolated polynucleotide encoding a non-natural fatty acyl-ACP thioesterase wherein the non-natural thioesterase has at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 1 and comprises:

i) Tyrosine (Y) or phenylalanine (F) at the position corresponding to position 163 of SEQ ID NO: 1 and lysine (K), or alanine (A) at the position corresponding to position 186 of SEQ ID NO: 1; or

ii) Phenylalanine (F) at the position corresponding to position 163 of SEQ ID NO: 1 and/or proline (P), lysine (K), or alanine (A) at the position corresponding to position 186 of SEQ ID NO: 1, wherein the non-natural thioesterase catalyzes the production of increased levels of C8:0 and C10:0 fatty acids in comparison to a wild-type thioesterase.

8. The isolated polynucleotide of claim 7, wherein the polynucleotide encodes a non-natural thioesterase further comprising a lysine (K) at the position corresponding to position 228 of SEQ ID NO: 1.

9. An expression cassette comprising the isolated polynucleotide of claim 7.

10. A host cell comprising the expression cassette of claim 9.

11. The host cell of claim 10, wherein the cell is an oleaginous microalga cell.

12. The host cell of claim 11, wherein the microalga cell is classified as Chlorophyta, Trebouxiophyceae, Chlorellales, Chlorellaceae, or Chlorophyceae.

13. The host cell of claim 11, wherein the microalga cell is of a genus selected from the group consisting of *Chlorella*, *Dunaliella*, and *Prototheca*.

14. The host cell of claim 9, wherein the microalga cell is of a species selected from the group consisting of *Prototheca moriformis* and *Chlorella protothecoides*.

* * * * *